STUDIES ON NEW ANTIBIOTIC LIVIDOMYCINS. V

IN VITRO AND *IN VIVO* ANTIMICROBIAL ACTIVITY OF LIVIDOMYCIN A

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In vitro and *in vivo* antimicrobial activities of lividomycin A were investigated. This substance showed a wide range of antimicrobial activity against most of Gram-positive bacteria including *Mycobacterium tuberculosis* and was also effective against Gram-negative bacteria including *Pseudomonas aeruginosa*, but was ineffective for streptococci, diplococci and fungi. The *in vitro* antimicrobial activity of lividomycin A was the greatest in media of pH 7.8. The minimum inhibitory concentration (MIC) was affected by inoculum size, but the addition of serum caused only slight fluctuation of MIC. *In vitro* development of resistance to lividomycin A in *P. aeruginosa* and *M. tuberculosis* was much slower than that to kanamycin, but was comparable in *Staphylococcus aureus*. In resistant mutants developed *in vitro*, cross resistance was observed among lividomycin A, kanamycin and gentamicin. In clinical isolates, however, no distinct cross resistance was found among these three antibiotics. Lividomycin A showed a positive protecting effect for the experimental infections in mice with several bacteria such as *S. aureus*, *P. aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*. It was fairly effective for the experimental infection with the kanamycin-resistant strains of *E. coli* and *P. aeruginosa* producing the kanamycin-phosphorylating enzyme.

It was reported previously that new aminoglycosidic antibiotics, lividomycins A and B, were produced from the culture broth of *Streptomyces lividus*, always being accompanied by the production of paromomycin and No. 2230-C (mannosylparomomycin)[1,2]. Lividomycin A is a pentasaccharide containing mannose, neosamine B, ribose and 3'-deoxyparomamine whose chemical structure was reported by ODA *et al.*[3,4]

This paper deals chiefly with the *in vitro* and *in vivo* activities of lividomycin A against Gram-positive and Gram-negative bacteria in comparison with related aminoglycoside antibiotics.

Materials and Methods

Antibiotics. Lividomycin A was prepared in this laboratory, Kowa Co., Ltd. The

other antibiotics such as kanamycin, streptomycin, gentamicin and paromomycin were purchased from commercial source.

Bacterial strains used. Standard strains of bacteria from our laboratory were used for the experiments. The clinical isolates of various species of bacteria were supplied from several hospitals in Tokyo. These strains were kept on heart infusion agar slants and subcultured on heart infusion agar plate before the experiments. Heart infusion agar containing 10% horse blood was used for the cultivation of diplococci, streptococci, *Hemophilus*, *Bordetella* and *Corynebacterium*, and 1% OGAWA's egg medium for *Mycobacterium*. Heart infusion agar containing 3% NaCl was used for *Vibrio parahaemolyticus*. For fungi, 2% glucose SABOURAUD's agar was used.

Antimicrobial activity test. Estimation of the antimicrobial activity of antibiotics against Gram-positive and Gram-negative bacteria, except for *Mycobacterium*, was carried out according to the two-fold serial agar dilution method using heart infusion agar (Eiken) with or without 10% horse blood. For *V. parahaemolyticus* heart infusion agar containing 3% NaCl was used as test medium. One loopful of an overnight Trypto-soy broth culture of each test organism (about 10^8 cells/ml) was streaked on each assay medium containing graded concentration of test antibiotic. For *Mycobacterium tuberculosis* and *Mycobacterium* 607, KIRCHNER's liquid medium containing 10% calf serum and heart infusion broth containing 1% glycerol were used respectively. Cells of *M. tuberculosis* were suspended in saline at the concentration of 1 mg/ml and 10^{-2} mg of the organism was inoculated in the test medium. For fungi, 2% glucose SABOURAUD's agar was adopted and fungi were suspended in saline containing 0.5% Tween 80 (3×10^6 spores per ml) and one loopful of the suspension was streaked on the assay plate.

Minimum inhibitory concentrations were determined after 24-hour incubation at 37°C for the majority of Gram-positive and Gram-negative bacteria except for several species described below and after 1 week incubation at 27°C for fungi. The MICs for *Bordetella*, *Hemophilus* and *Mycobacterium* 607 were determined after 48-hour incubation at 37°C and that for *M. tuberculosis* was estimated after 3-week incubation at 37°C.

Bactericidal activity test. The bactericidal activity of lividomycin A was estimated against *S. aureus* and *P. aeruginosa* in both saline containing 0.25% casamino acids (pH 7.2) at 20°C and heart infusion broth (Difco) at 37°C with shaking. Aliquots of the solution were taken at appropriate intervals and the sample was diluted with saline containing 0.25% casamino acids. One ml of each diluent was placed in Petri dishes, mixed well with poured melted nutrient agar. Viable cell count was conducted after 48-hour incubation.

Development of resistance. The rate of the development of resistance to lividomycin A and kanamycin was studied using *S. aureus*, *P. aeruginosa* and *M. tuberculosis*. The former two strains were cultivated at 37°C for 48 hours in heart infusion broth containing several concentrations of antibiotics and one loopful of the culture permitting the growth and containing the highest level of test drug was transferred to be subcultured into heart infusion broth containing the higher concentrations of the antibiotic. *M. tuberculosis* was cultivated for 3 weeks in KIRCHNER's liquid medium containing antibiotics. The same procedure described above was conducted repeatedly.

Binding with serum protein. Lividomycin A or other aminoglycosidic antibiotics were dissolved with M/15 phosphate buffer (pH 7.4) containing 1% horse serum at the concentration of 1 mg/ml. After 20-hour incubation at 4°C, the solution was centrifuged at $200{,}000\times g$ for 4.5 hours. The concentration of antibiotics in the supernatant fluid was determined by a paper disk method using *Bacillus subtilis* PCI 219 as the test organism.

Experimental infection in mice. ICR-JCL male mice, 4 weeks old and weighing 18~22 g were used. Ten mice per each experimental group were challenged intraperitoneally with 0.4 ml of bacterial suspension such as *E. coli*, *P. aeruginosa*, *S. aureus*, *Streptococcus haemolyticus* and *K. pneumoniae* with or without 4% mucin. Test antibiotic was given

once at 2 hours after challenge and mice were observed for 1 week. Effective doses (ED_{50}) of antibiotics were calculated by VAN DER WAERDEN's method[5].

Results

Antimicrobial activity

Lividomycin A, as shown in Table 1, had a wide antimicrobial spectrum against Gram-positive bacteria including *M. tuberculosis* and was also effective against Gram-negative bacteria including *P. aeruginosa*; the MICs (mcg/ml) were 1.56 for *M. tuberculosis*, 3.13~6.25 for *S. aureus*, 12.5~50 for *P. aeruginosa* and 6.25~25 for enterobacteria such as *E. coli*, *Proteus*, *Klebsiella*, *Shigella* and *Salmonella*. It was

Table 1. Antimicrobial spectra of lividomycin A and kanamycin

Microorganism	Medium	MIC (mcg/ml)		Microorganism	Medium	MIC (mcg/ml)	
		Lividomycin A	Kanamycin			Lividomycin	Kanamycin
Staphylococcus aureus FDA 209P JC-3	HIA	6.23	3.13	*Salmonella paratyphi* B	HIA	12.5	6.25
				Salmonella typhi-murium	HIA	25	6.25
Staphylococcus aureus Smith	HIA	3.13	1.56	*Shigella flexneri* 2a	HIA	12.5	6.25
				Shigella flexneri 3a	HIA	25	6.25
Staphylococcus aureus Newman	HIA	3.13	6.25	*Shigella sonnei*	HIA	12.5	12.5
Staphylococcus epidermidis STP-19	HIA	0.78	0.78	*Klebsiella pneumoniae* PCI-602	HIA	6.25	3.13
Micrococcus flavus M-16	HIA	>100	6.25	*Proteus vulgaris* OX-19	HIA	3.13	12.5
Sarcina lutea PCI-1001	HIA	50	3.13	*Pseudomonas aeruginosa* A_3	HIA	12.5	100
Bacillus subtilis PCI-219	HIA	0.39	0.20				
Bacillus cereus	HIA	6.25	6.25	*Pseudomonas aeruginosa* $\bar{O}i$	HIA	50	100
Bacillus anthracis	HIA	3.13	6.25	*Vibrio parahaemolyticus* 1648	N-HIA	>100	50
Corynebacterium diphtheriae Yanagisawa	B-HIA	0.78	0.78				
Corynebacterium diphtheriae Ohara	B-HIA	0.78	0.78	*Mycobacterium tuberculosis* $H_{37}Rv$	KR	1.56	1.56
				Mycobacterium 607	GB	0.78	0.39
Corynebacterium xerosis 53-K-I	B-HIA	0.39	0.39	*Candida albicans*	SG	>200	>200
				Candida krusei	SG	>200	>200
Streptococcus haemolyticus Cook	B-HIA	>100	50	*Candida parakrusei*	SG	>200	>200
Streptococcus haemolyticus S-8	B-HIA	50	25	*Candida tropicalis*	SG	>200	>200
				Candida pseudotropicalis	SG	>200	>200
Streptococcus faecalis Imanari	B-HIA	>100	50	*Candida guilliermondi*	SG	>200	>200
				Candida stellatoidea	SG	>200	>200
Diplococcus pneumoniae type I	B-HIA	>100	100	*Saccharomyces cerevisiae*	SG	>200	>200
Diplococcus pneumoniae type II	B-HIA	>100	100	*Cryptococcus neoformans*	SG	>200	>200
				Trychophyton asteroides	SG	>200	>200
Haemophilus influenzae Shiga	B-HIA	25	6.25	*Trychophyton interdigitale*	SG	>200	>200
Bordetella pertussis Tōhama	B-HIA	25	6.25	*Trychophyton rubrum*	SG	>200	>200
Escherichia coli NIHJ	HIA	25	12.5	*Microsporium gypseum*	SG	>200	>200
Escherichia coli O-26	HIA	25	12.5	*Aspergillus fumigatus*	SG	>200	>200
Escherichia coli O-55	HIA	12.5	6.25	*Aspergillus niger*	SG	>200	>200
Salmonella typhosa H-901	HIA	6.25	3.13	*Aspergillus flavus*	SG	>200	>200
Salmonella paratyphi A	HIA	6.25	3.13	*Penicillium frequentas*	SG	>200	>200

Note: HIA: Heart infusion agar. B-HIA: Heart infusion agar containing 10% blood. N-HIA: Heart infusion agar containing 3% NaCl. KR: KIRCHNER medium containing 10% calf serum. GB: Heart infusion broth containing 1% glycerin. SG: SABOURAUD glucose agar.

ineffective to *Micrococcus flavus*, *Streptococcus pyogenes*, *S. faecalis*, *Diplococcus pneumoniae*, *V. parahaemolyticus* and fungi. According to our experimental results, the antimicrobial activity of lividomycin A was nearly similar to that of kanamycin, although the MIC values of the former were generally somewhat greater than the latter, while the activity of lividomycin A for *P. aeruginosa* was greater than that of kanamycin.

Influence of inoculum size, the reaction of test medium and the addition of serum on the activity of lividomycin A

The activity of lividomycin A (Table 2), was apparently affected by inoculum size, and the degree of influence seems to be larger in *S. aureus* than in *P. aeruginosa*. The greatest activity of lividomycin A was in test media at pH 7.8 against the organisms tested and the activity was progressively reduced along with the decrease in pH value (Table 3). Except for 2 strains of *S. aureus*, the activity was not affected by the addition of 10% horse serum into test media but the addition of

Table 2. The influence of inoculum size on the antimicrobial activity of lividomycin A against *S. aureus* and *P. aeruginosa*

Strain	Inoculum size (cells/ml of inoculum suspension)	MIC (mcg/ml)
Staphylococcus aureus FDA 209 P	1.5×10^{9}	12.5
	1.5×10^{8}	6.25
	1.5×10^{7}	1.56
	1.5×10^{6}	0.78
Pseudomonas aeruginosa Ka-2	3.3×10^{8}	25
	3.3×10^{7}	25
	3.3×10^{6}	25
	3.3×10^{5}	12.5

Table 3. The influence of pH of test medium on antimicrobial activity of lividomycin A

Organism	pH				
	6.0	6.6	7.2	7.8	8.4
E. coli NIHJ	100	50	25	25	25
S. flexneri 2b EW-40	50	25	12.5	6.25	6.25
K. pneumoniae PCI-602	50	12.5	6.25	3.13	6.25
P. aeruginosa A_3	25	12.5	6.25	3.13	6.25
B. subtilis PCI-219	1.56	1.56	0.78	0.78	3.13
S. aureus Newman	25	12.5	12.5	6.25	25
S. epidermidis	1.56	1.56	0.78	0.78	3.13

* Each figure indicates minimum inhibitory concentration in terms of mcg per ml.

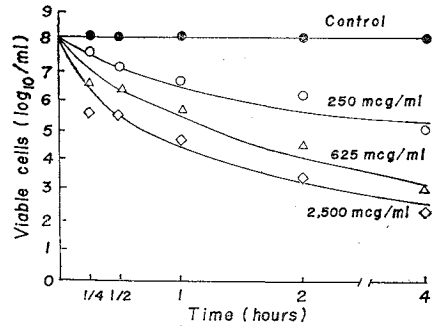
Table 4. The influence of the addition of horse serum to the test medium on antimicrobial activity of lividomycin A

Organism	Serum (%)				
	None	5	10	20	50
E. coli NIHJ	25	25	25	50	50
S. flexneri 2b EW-40	12.5	12.5	12.5	25	25
K. pneumoniae PCI-602	6.25	6.25	6.25	12.5	12.5
P. aeruginosa A_3	6.25	6.25	6.25	12.5	12.5
B. subtilis PCI-219	0.78	0.78	0.78	0.78	0.78
S. aureus FDA 209P	6.25	25	50	50	50
S. aureus Newman	6.25	25	50	50	50
S. epidermidis STP-19	0.78	0.78	0.78	3.13	6.25

* Each figure indicates minimum inhibitory concentration in terms of mcg per ml.

Fig. 1. Bactericidal activity of lividomycin A against *P. aeruginosa* A_3.

Determination of bactericidal activity was carried out in physiological saline containing 0.25% casamino acids at 20℃.



more than 20% serum caused slight increase in MIC values (Table 4).

Binding with serum protein

The rate of binding of lividomycin A with horse serum protein was determined in comparison with other aminoglycosidic antibiotics. Lividomycin A bound with serum protein at 11.5% rate, being approximately similar to that of kanamycin (10.0%), neomycin (8.0%) and paromomycin (11.5%).

Bactericidal activity

Bactericidal activity of lividomycin A against *P. aeruginosa* in saline containing casamino acids was tested and the result was shown in Fig. 1. In saline without the antibiotic, the number of viable cells remained unchanged after 4-hour incubation at 20°C, whereas the number was reduced at 10^{-3} by the addition of 250 mcg/ml lividomycin A, 10^{-5} by 625 mcg/ml and 10^{-6} by 2,500 mcg/ml, respectively. On the other hand, they were not affected even by the addition of 10 mg/ml kanamycin and 20 mg/ml paromomycin.

Fig. 2. Bactericidal activity of lividomycin A against *P. aeruginosa* A_3 in brain heart infusion broth.

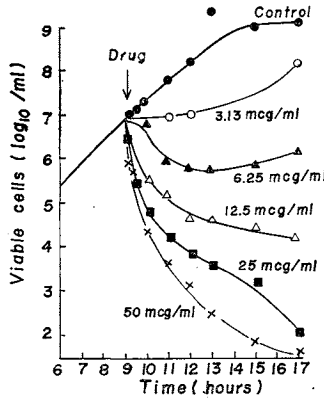


Fig. 3. Bactericidal activity of kanamycin against *P. aeruginosa* A_3 in brain heart infusion broth.

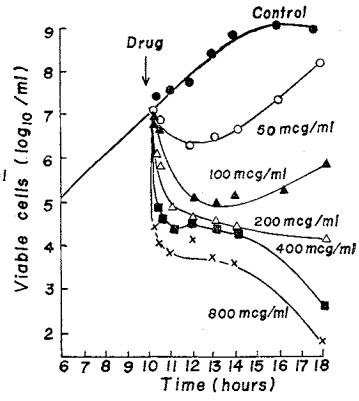


Fig. 4. Bactericidal activity of lividomycin A against *S. aureus* FDA 209P in brain heart infusion broth.

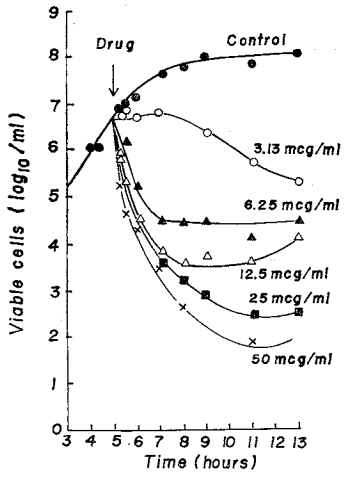
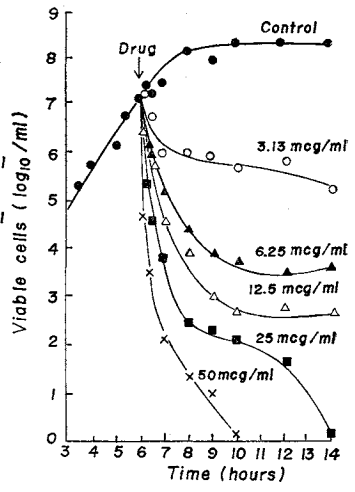


Fig. 5. Bactericidal activtiy of kanamycin against *S. aureus* FDA 209P in brain heart infusion broth.



Bactericidal activity of lividomycin A was also tested in heart infusion broth as compared with that of kanamycin. Cell proliferation was slightly inhibited at 3.13 mcg/ml lividomycin A and at the concentration of above 6.25 mcg/ml, the bacterial growth was progressively inhibited along with the increase in the concentration of antibiotic (Fig. 2). Kanamycin showed a bactericidal activity as similar to that of lividomycin A at approximately 16 fold higher concentrations (Fig. 3).

In *S. aureus*, lividomycin A showed stronger bactericidal activity than that in *P. aeruginosa*. The activity, however, was somewhat weaker than that of kanamycin in both media (Figs. 4 and 5).

In vitro Development of resistance and cross resistance

The pattern of acquisition of drug resistance against lividomycin A was investigated in comparison with both kanamycin and gentamicin. The progression and degree of resistance of three test organisms are shown in Fig. 6. The rate of development of resistance of lividomycin A was much slower than that of kanamycin in *P. aeruginosa* and *M. tuberculosis*, while it was comparable in *S. aureus*. No significant difference was shown between lividomycin A and gentamicin.

The lividomycin A-resistant strain of *S. aureus* which was artificially developed *in vitro* showed a high resistance to kanamycin and a moderate resistance to streptomycin and gentamicin. The kanamycin-resistant strain and the gentamicin-resistant strain also showed high resistance to lividomycin A, whereas the streptomycin-resistant strain remained sensitive against lividomycin A and kanamycin (Table 5). A similar result was also observed in *P. aeruginosa* (Table 6).

Fig. 6. Patterns of *in vitro* developments of resistance of three test organisms to lividomycin A and kanamycin.

The ordinate indicates the maximum drug concentration that bacterial growth is allowed, and the abscissa the number of test tube transfers.

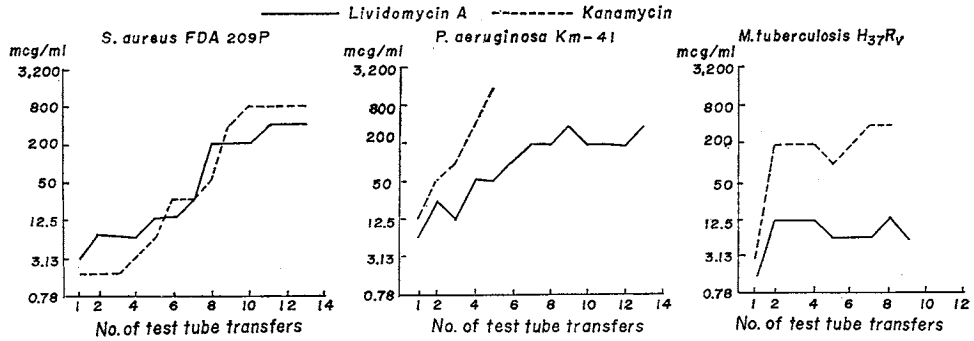


Table 5. Cross resistance patterns of artificially-induced resistant strains of *S. aureus* FDA 209P among lividomycin A and related antibiotics.

Strain	MIC (mcg/ml)			
	Lividomycin A	Kanamycin	Streptomycin	Gentamicin
S. aureus FDA 209P	6.25	3.13	3.13	0.39
S. aureus FDA 209P LVM-R	800	400	50	25
S. aureus FDA 209P KM-R	400	100	50	12.5
S. aureus FDA 209P SM-R	12.5	12.5	1,600	0.78
S. aureus FDA 209P GM-R	800	400	25	25

Abbreviations: LVM: lividomycin A, KM: kanamycin, SM: streptomycin, GM: gentamicin.

Table 6. Cross resistance patterns of artificially-induced resistant strains of *P. aeruginosa* Km-41 among lividomycin A and related antibiotics.

Strain	MIC (mcg/ml)			
	Lividomycin A	Kanamycin	Gentamicin	Colistin
P. aeruginosa Km-41	50	100	3.13	3.13
P. aeruginosa LVM-R	>200	1,600	100	6.25
P. aeruginosa KM-R	>200	1,600	>200	6.25
P. aeruginosa GM-R	>200	800	25	12.5
P. aeruginosa CL-R	50	50	6.25	200

Abbreviations: LVM: lividomycin A, KM: kanamycin, GM: gentamicin, CL: colistin.

Table 7. Cross resistance patterns between lividomycin A and related antibiotics in fresh, naturally-resistant strains

Strain	MIC (mcg/ml)			
	Lividomycin A	Kanamycin	Gentamicin	Streptomycin
P. aeruginosa GM, SM-R	12.5	100	>200	>800
GM, KM, SM-R	12.5	200	>200	>800
KM-R	12.5	>800	3.13	50
KM, SM-R	12.5	>800	12.5	>800
KM, SM, LVM-R	>800	>800	3.13	>800
E. coli KM, SM, LVM-R	>800	>800	0.4	>800
KM, SM-R	12.5	>800	0.4	400
K. pneumoniae KM, SM, LVM-R	>800	>800	0.4	>800
KM, SM, GM-R	6.3	>800	25	100

Abbreviations: LVM: lividomycin A, KM: kanamycin, SM: streptomycin, GM: gentamicin.

Table 8. Susceptibility of clinical isolates to lividomycin A

Strain	No. of strain tested	MIC of lividomycin A										
		≧200	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.2
S. aureus	134	36	1	1	8	59	25	1	1	2		
P. aeruginosa	215	14	15	69	84	22	5	3	3			
E. coli	52	1		1	8	31	4	6	1			
Salmonella group	50		2		31	15	2					
Shigella group	48				11	34	1	2				
V. parahaemoliticus	49	33	15			1						
M. tuberculosis	39	1	2			3	10	3	3	8	4	5

* Each figure indicates the number of strain which showed an appropriate MIC.

Table 9. Therapeutic effects of lividomycin A and kanamycin for experimental infections in mice.

Strain	Challenge doses (cells/mouse)	Mucin[a]	Route	ED_{50} (confidence limits, p=0.05) mg/kg	
				Lividomycin A	Kanamycin
S. aureus Smith	1.2×10^4 (100 MLD)	+	p. o.	233 (325~167)	154 (189~126)
			i. p.	0.313 (0.407~0.241)	0.156 (0.238~0.102)
			s. c.	2.30 (2.83~1.87)	1.25 (1.81~0.863)
P. aeruginosa NC-5	3.5×10^4 (100 MLD)	+	i. p.	10.16 (14.13~6.92)	50.0 (64.0~39.0)
			s. c.	40.6 (57.7~28.6)	81.3 (113~74.0)
			i. m.	61.5 (90.4~41.9)	75.9 (104~55.3)
P. aeruginosa TK-157 [b]	6.0×10^4 (1 MLD)	+	i. p.	100 (144.3~69.3)	>800
P. aeruginosa TI-13 [c] (KM-R, LVM-R)	6.8×10^4 (1 MLD)	+	i. p.	>400	>800
S. haemolyticus S-23	1.1×10^3 (10 MLD)	−	i. p.	>400	170.1 (251~115)
K. pneumoniae 34	1.5×10^4 (10 MLD)	−	i. p.	4.74 (5.17~4.34)	<1.37
			s. c.	3.85 (4.00~3.70)	3.85 (5.30~2.79)
E. coli GN-2411	3.5×10^2 (1 MLD)	+	s. c.	46.7 (50.0~43.0)	16.5 (23.4~11.6)
E. coli GN-1970 [d] (KM-R)	1.2×10^5 (1 MLD)	+	s. c.	43.5 (56.3~33.6)	>400

Ten male mice (weighing 18~22 g) per each experimental group were challenged intraperitoneally with each bacterial suspension with or without 4% mucin. Antibiotic was given once at 2 hours after inoculation, except for the infection with *E. coli*. For the experimental infections with *E. coli*, drug was given twice at 2 and 7 hours after inoculation.

a) + with mucin. − without mucin.
b) The strain produces kanamycin-phosphorylating enzyme.
c) The strain produces kanamycin- and lividomycin-phosphorylating enzyme.
d) The strain also produces kanamycin-phosphorylating enzyme.

Therefore, in artificially-induced resistant mutants, an intimate cross resistance was found among lividomycin A, kanamycin and gentamicin, but not between lividomycin A and streptomycin. In clinical isolates, however, it was found that there existed lividomycin-sensitive strains among high kanamycin-resistant strains and gentamicin-resistant ones (Table 7).

Susceptibility of clinical isolates

Clinical isolates of various species of bacteria were investigated for their sensitivity to lividomycin A, and the result was summarised in Table 8.

Lividomycin A was found to inhibit 88 (66.5 %) of 134 strains of *S. aureus* at 12.5 mcg/ml or less, 186 (86.5 %) of 215 strains of *P. aeruginosa* at 50 mcg/ml or less, 42 (80.8 %) of 52 strains of *E. coli* at 12.5 mcg/ml or less, 34 (79.8 %) of 48 strains of *Shigella* at 12.5 mcg/ml or less and 48 (96.0 %) of 50 strains of *Salmonella* at 25 mcg/ml or less. Moreover, it inhibited 33 (84.6 %) of 39 strains of *M. tuberculosis* including 19 kanamycin-resistant strains at 6.25 mcg/ml or less.

Therapeutic effect against experimental infections in mice

The therapeutic effects of lividomycin A against experimental infections with several bacterial species were summarized in Table 9 in comparison with kanamycin.

For the experimental infection with *S. aureus*, *K. pneumoniae* and *E. coli*, the therapeutic activities of lividomycin A are nearly equal or slightly weaker than that of kanamycin, whereas the activity is higher than that of kanamycin for the infection with *P. aeruginosa* NC-5. Although kanamycin was quite ineffective against the infections with kanamycin-resistant strains of *E. coli* and *P. aeruginosa* which produced kanamycin-inactivating enzyme, lividomycin A showed protective activity against these infections. However, lividomycin A showed no effect for the infections with *S. haemolyticus* and lividomycin-resistant strain of *P. aeruginosa* producing kanamycin- and lividomycin-inactivating enzyme.

Discussion

According to the experimental results described above, the antibacterial activity of lividomycin A resembled closely that of kanamycin and paromomycin, except that this substance possessed a moderate activity against strains of *P. aeruginosa*.

Although an intimate cross resistance was noted between this antibiotic and others of the aminoglycoside group such as kanamycin and gentamicin with artificially-induced resistant cultures, such cross resistance was not always found in clinical isolates. As it is quite well known, kanamycin is inactivated by phosphorylating enzyme from the R factor-mediated multiple drug-resistant cultures of enterobacteria as well as pseudomonads resulting in the formation of 3′-phosphorylkanamycin[6~12]. This enzyme was shown to be active not only to kanamycin but also to aminodeoxykanamycin, neomycin and paromomycin, while gentamicin and lividomycin A were highly stable to this enzyme[13~14], presumably due to the fact that both of these substances were devoid of hydroxy group at C-3 position of D-aminoglucose moiety containing deoxystreptamine in each structure[4,15]. Such difference in the chemical structure of lividomycin A from other related substances may account for the fact that this antibiotic was effective in experimental infections in mice with kanamycin-resistant strains of *E. coli* and *P. aeruginosa* producing the kanamycin-phosphorylating enzyme.

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