STUDIES ON NEW ANTIBIOTIC LIVIDOMYCINS
VI. ABSORPTION, EXCRETION AND TISSUE DISTRIBUTION OF LIVIDOMYCIN A

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Studies on lividomycin A, a new broad-spectrum aminoglycosidic antibiotic, were conducted in rats, rabbits and dogs to determine its plasma levels, urinary and biliary excretion as well as tissue distribution. Intramuscular administration of lividomycin A resulted in high potentially therapeutic plasma concentration with concentration depending on the dose. Seventy to 90% of drug administered intramuscularly to dogs and rabbits was recovered in the urine primarily within 8 hours of dosing. In contrast only 2% of an oral dose was recovered in rabbit urine, indicating poor absorption from the gut. Biliary excretion was measurable but minimal. High concentrations of the antibiotic were observed in the plasma, lung and kidney of the rats, with slow clearance from the kidney. Thin-layer chromatography of dog urine gave no evidence for the presence of biologically active metabolites. All studies indicated that lividomycin A behaved similarly to kanamycin.

The structure, physicochemical properties and biological activities of lividomycin A have been reported in previous papers.[1~4] Lividomycin A is a new aminoglycosidic antibiotic containing 2-amino-2,3-dideoxy-D-glucose that was isolated from culture broths of *Streptomyces lividus* nov. sp. The antibiotic was identified as mannosyldeoxyparomomycin and was found active against a wide range of Gram-positive and Gram-negative bacteria.

This paper described studies on the absorption, excretion and tissue distribution of lividomycin A in rabbits, dogs and rats.

Materials and Methods

Antibiotics: Lividomycin A sulfate and commercial kanamycin sulfate (Meiji Seika Kaisha, Ltd.), the reference drug, were used with the amounts expressed as the weight of free base.

Animals: The animals used included male rabbits weighing 2~3 kg, male dogs (mongrel) weighing 10~15 kg and male Sprague Dawley rats weighing 130~170 g. All animals were fasted overnight before use.

Determination of Lividomycin A and Kanamycin Concentrations: The concentration of lividomycin A and kanamycin in plasma, urine, bile and tissue were determined by the disc method, with the exception those in plasma of dogs were determined by the cup method. In both procedures 100 ml of melted Streptomycin Assay Agar (Difco, pH 7.8) was inoculated with 0.3 ml of a spore suspension (3×10^8 spores per ml) of *Bacillus subtilis*

ATCC 6633. The diameters of inhibitory zones were measured after diffusion at 5°C for 3 hours and incubation at 37°C for 18 hours. The amounts of each drug in the test samples were calculated from the appropriate standard curve. Standard antibiotic solutions used in the preparation of the standard curve were obtained by dissolving and diluting with plasma (for determination of levels in plasma), dissolving and diluting with 1/15 M phosphate buffer solution at pH 8 (for determination of levels in urine and tissues), or dissolving and diluting with bile (for determination of levels in bile of dogs).

Plasma Levels and Urine Excretion in Rabbits: Either lividomycin A or kanamycin dissolved in distilled water was administered intramuscularly at a single dose of 50 mg/kg to rabbits. After administration of the antibiotic, blood was withdrawn from the auricle vein at intervals over a period of 24 hours. The blood was allowed to clot and the plasma separated for bioassay. Urine samples were collected in polyethylene tubes inserted in the ureter at the specified times. In addition, lividomycin A and kanamycin were given to individual rabbits at a single oral dose of 500 mg/kg. Plasma and urine levels were determined by the procedures described.

Plasma Levels and Urine Excretions in Dogs: The following experiments were conducted by using the same 3 dogs: First, after administering 20 mg/kg of kanamycin, dissolved in distilled water, intramuscularly to the dogs, blood samples were collected from the cephalic vein, and urine samples by catheterization at specified times over a period of 24 hours after administration. Secondly, the same dogs were dosed in the same manner with 20 mg/kg, 40 mg/kg or 80 mg/kg of lividomycin A to all as a single dose. Each dog was dosed with each of the three antibiotic concentrations at intervals of one week. Finally the following experiment was conducted for the purpose of comparing the plasma and urine levels observed between normal dogs and those that had experimental acute renal failure. Renal failure was produced by giving an intravenous injection of 3 mg/kg of 1% mercuric chloride, dissolved in a physiological saline solution. Lividomycin A, 20 mg/kg, was administered intramuscularly 24 hours later and the blood and urine samples were collected and processed.

Chromatography of Dog Urine: The urine excreted by one dog to which lividomycin A was administered intramuscularly at a single dose of 20 mg/kg, was collected over a period of 24 hours after administration and examined by bioautography in combination with thin-layer chromatography. For control purpose, lividomycin A (10~20 mcg) was dissolved in the urine of a control dog. The urine sample (0.01 ml) was developed by thin-layer chromatography on a silica-gel plate using the upper layer of chloroform - methanol - 17% ammonia (2 : 1 : 1). Then, the plate was set on agar previously seeded with *Bicillus subtilis* ATCC 6633.

Biliary Excretion in Dogs: Dogs were previously anesthetized with 25 mg/kg of nembutal and canulated with a polyethylene tube inserted in the common bile duct. Lividomycin A was administered to the dogs intramuscularly at a single dose of 50 mg/kg. Blood, urine and bile samples were collected at intervals and the concentration of antibiotic in the bile was determined by bioassay.

Tissue Distribution in Rats: Lividomycin A dissolved in distilled water was administered intramuscularly to 25 rats at a single dose of 50 mg/kg. At 0.5, 1, 3, 5 and 12 hours after administration, 5 rats in each group were sacrificed by cutting the carotids and removing the blood. The brain, liver, kidney, lung, heart, muscle and spleen were removed, washed with physiological saline solution and homogenized with 4-fold volumes of 1/15 M phosphate buffer solution (pH 8). The homogenates were centrifuged at 3,000 rpm for 20 minutes and the supernatants were bioassayed. Blood samples were also collected for determination of lividomycin A.

Results and Discussion

1. Plasma Levels in Rabbits, Dogs and Rats

When lividomycin A or kanamycin was administered intramuscularly to rabbits at 50 mg/kg, the level of lividomycin A in the plasma reached a mean maximum value of 87.7 mcg/ml 30 minutes later, compared to 93.5 mcg/ml for kanamycin (Table 1). The plasma levels of both antibiotics declined gradually from their peaks and no antibiotic activity was found in the plasma 24 hours after administration of either antibiotic. The half life of lividomycin A (1.7 hour) was somewhat longer than that of kanamycin.

The results of the cross-over experiment in dogs are summarized in Table 2. Maximum plasma levels were observed one hour after administration of either lividomycin A or kanamycin. The peak concentrations, achieved with lividomycin A, were 34.3 mg/ml at 20 mg/kg, 53.7 mcg/ml at 40 mg/kg and 86.0 mcg/ml at 80 mg/kg. In comparison, the administration of kanamycin at 20 mg/kg gave a maximum concentration in plasma of 23.6 mcg/ml. The levels of the antibiotic in the plasma decreased gradually with the passage of time, disappearing completely 24 hours after administration. It should be noted that the peak concentration of antibiotic may well have occurred prior to the first sampling time of one hour. According to experiments with both rabbits and rats (to be discussed next), a maximum concentration of both lividomycin A and kanamycin was obtained 30 minutes after injection. The half life of lividomycin A increased with dosage from 1.2 hour for 20 mg/kg, to 1.3 hour for 40 mg/kg and to 1.7 hour for 80 mg/kg. When lividomycin A was administered at

Table 1. Plasma levels after intramuscular administration of a single dose (50 mg/kg) of lividomycin A and kanamycin in rabbits

Antibiotics (Number of animals)		Plasma level (mcg/ml)												Half life hour
		5 min.	15 min.	30 min.	45 min.	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.	8 hrs.	24 hrs.	
Lividomycin A (3)	Mean	42.3	65.7	87.7	71.0	48.8	41.5	31.5	19.4	12.8	10.7	5.7	3.1	—	1.7
	S.E.*	±10.9	±12.8	±21.4	±14.6	±6.1	±7.0	±7.9	±6.1	±5.6	±6.4	±3.1	±1.7		
Kanamycin (2)	Mean	28.0	67.0	93.5	68.0	67.0	34.5	19.1	10.5	7.5	5.0	2.6	2.6	—	1.2

Table 2. Plasma levels after intramuscular administration of a single dose of lividomycin A and kanamycin in dogs

Antibiotics (Number of animals)	Dose (mg/kg)		Plasma level (mcg/ml) 1 hr.	2 hrs.	4 hrs.	6 hrs.	8 hrs.	24 hrs.	Half life hour
Lividomycin A (3)	20	Mean	34.3	23.0	7.1	1.9	0.6	—	1.2
		S.E.*	±4.6	±2.0	± 1.6	± 0.5	± 0.1		
	40	Mean	53.7	38.7	13.7	3.2	1.1	—	1.3
		S.E.	±4.5	±2.9	± 3.1	± 0.7	± 0.4		
	80	Mean	86.0	50.3	31.0	14.3	4.8	—	.6
		S.E.	±4.0	±2.2	± 2.3	± 1.3	± 0.3		
	$HgCl_2$+ 20	Mean	63.6	54.0	53.7	48.7	42.3	25.7	
		S.E.	±9.6	±5.3	±10.7	±13.9	±11.1	±14.8	
Kanamycin (3)	20	Mean	23.7	12.0	2.9	0.5	trace	—	1.0
		S.E.	±4.1	±0.3	±0.5	± 0.2			

* S.E. = Standard error

20 mg/kg under conditions of acute renal failure brought on by mercuric chloride, the maximum plasma level reached 63.3 mcg/ml (mean value) one hour after administration, a value twice that observed under normal conditions. The plasma level of lividomycin A remained high and was still at 25.7 mcg/ml (mean value) 24 hours after administration of the antibiotic.

As shown in Table 7, when lividomycin A was administered intramuscularly to rats at a single dose of 50 mg/kg, the maximum plasma concentration was 128.3 mcg/ml (mean value) 30 minutes after administration. However, the plasma level declined rapidly with little antibiotic activity remaining in the 3 hour plasma.

In order to compare lividomycin A and kanamycin by the oral route, a single dose of 500 mg/kg of each agent was administered by mouth to rabbits with the results shown in Table 3. The plasma level of lividomycin A reached a maximum value of 4 mcg/ml 2 hours after administration, one fifth that for kanamycin. This result indicates that lividomycin A is minimally absorbed when given by mouth, at least in this species.

2. Urinary Excretion in Rabbits and Dogs

The concentrations and recovery of antibiotics were determined in the urine of rabbits and dogs after intramuscular administration, and in the urine of rabbits after oral administration with the results given in Tables 3, 4 and 5.

Over 90 % recovery of antibiotic was obtained in 24-hour urine samples or rabbits after intramuscular administration of 50 mg/kg of either lividomycin A or kanamycin. In dogs, recovery varied with dosage of lividomycin A as follows: 95.5 % for 20 mg/kg, 82.3 % for 40 mg/kg and 87.0 % for 80 mg/kg of lividomycin A, compared to 85.3 % for 20 mg/kg of kanamycin. Urinary concentrations, in excess of 1,000 mcg/ml,

Table 3. Plasma levels and urinary excretions after oral administration of a single dose (500 mg/kg) of lividomycin A and kanamycin in rabbits

Antibiotics (Number of animals)		Plasma level (mcg/ml) 0.5 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.	8 hrs.	12 hrs.	24 hrs.	Urine recovery 0~24 hrs. %
Lividomycin A (5)	Mean	0.6	2.2	4.6	3.5	1.3	0.7	0.2	0.2	2.2
	S.E.	±0.3	±1.2	±2.3	±1.4	±0.6	±0.6	±0.2	±0.2	±0.7
Kanamycin (2)	Mean	3.0	10.7	16.0	21.0	17.9	6.8	4.7	0.8	3.3

Table 4. Urinary excretions after intramuscular administration of a single dose (50 mg/kg) of lividomycin A and kanamycin in rabbits

Antibiotics (Number of animals)		0~1/2 hr.	1/2~1 hr.	1~2 hrs.	2~3 hrs.	3~4 hrs.	4~5 hrs.	5~7 hrs.	7~8 hrs.	8~24 hrs.
Lividomycin (3) 2.8~3.1 kg	Urine level (mcg/ml)	2,600 ~ 5,100	6,000 ~ 7,100	5,800 ~ 10,100	5,200 ~ 8,500	5,400 ~ 6,000	1,600 ~ 8,100	410 ~ 600	130 ~ 400	10 ~ 80
	Recovery mean (%) S.E.			42.0 ±2.7	62.8 ±2.3	74.9 ±2.8	83.3 ±1.7	87.8 ±3.1	89.1 ±3.3	90.6 ±3.7
Kanamycin (2) 2.5~2.55 kg	Urine level (mcg/ml)	480 ~ 640	900 ~ 23,000	4,800 ~ 13,000	3,300 ~ 18,500	7,400 ~ 50,000	4,700 ~ 10,000	1,200 ~ 7,100	1,500 ~ 2,270	26 ~ 300
	Recovery mean (%)			15.7	31.7	62.7	74.2	81.7	83.9	95.1

Table 5. Urinary excretion after intramuscular admininistration of a single dose of lividomycin A and kanamycin in dogs

Antibiotics (Number of animals)	Dose (mg/kg)		0~2 hrs.	2~4 hrs.	4~6 hrs.	6~8 hrs.	8~24 hrs.
Lividomycin A (3)	20 (10.8~14.3 kg)	Urine level (mcg/ml)	740~ 3,400	1,600~ 4,100	520~ 1,800	620~ 950	21~65
		Recovery mean (%) S.E.	59.2 ±9.8	79.1 ±6.9	85.5 ±6.5	92.8 ±3.3	95.5 ±3.2
	40 (10.8~14.5 kg)	Urine level (mcg/ml)	5,600~ 15,300	3,000~ 13,000	1,350~ 6,800	260~ 1,800	54~120
		Recovery mean (%) S.E.	45.5 ±9.3	67.6 ±6.7	78.0 ±9.0	79.7 ±9.6	82.3 ±9.4
	80 (10.9~14.5 kg)	Urine level (mcg/ml)	11,300~ 23,000	7,400~ 13,000	1,400~ 13,000	140~ 1,000	105~240
		Recovery mean (%) S.E.	49.0 ±1.9	72.3 ±3.5	82.2 ±1.8	83.4 ±2.0	87.0 ±2.1
	$HgCl_2$+20 (9.8~13.9 kg)	Urine level (mcg/ml)	460± 1,200	250~ 1,300	125~ 1,300	320~ 570	125~ 470
		Recovery mean (%) S.E.	7.0 ±0.6	11.3 ±0.5	16.0 ±3.0	18.0 ±3.6	27.1 ±7.4
Kanamycin (3)	20 (10.0~12.3 kg)	Urine level (mcg/ml)	820~ 8,400	2,300~ 3,700	500~ 1,150	150~ 250	14~18
		Recovery mean (%) S.E.	54.8 ±5.9	77.3 ±6.1	82.7 ±6.5	84.3 ±6.6	85.3 ±6.6

Table 6. Biliary excretion, urinary excretion and plasma level after intramuscular administration of a single dose (50 mg/kg) of lividomycin A in dogs

3 Dogs	Bile concentration (mcg/ml)										Urine recovery 0~24 hrs. %
	30 min.	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.	8 hrs.	24 hrs.	
Mean	2.2	6.7	8.7	7.3	2.2	2.0	0.6	0.4	—	—	74.1
S.E.	±1.3	±3.2	±5.2	±4.9	±2.2	±2.0	±0.6	±0.4			±10.3

3 Dogs	Plasma level (mcg/ml)													Half life hrs.
	5 min.	15 min.	30 min.	45 min.	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.	8 hrs.	24 hrs.	
Mean	9.6	29.5	52.7	57.3	59.0	48.3	29.3	28.3	18.8	14.7	13.9	8.2	—	2.1
S.E.	±0.6	±9.1	±19.7	±13.6	±20.0	±12.9	±9.9	±7.4	±4.6	±5.7	±5.5	±3.4		

continued for 6 hours after administration of lividomycin A and for 4 hours after administration of kanamycin to dogs. Similar result was also obtained in rabbits.

On administration of 20 mg/kg to dogs under acute renal failure by mercuric chloride, 27.1 % of lividomycin A was recovered in the 24-hour urine. The recovery rate of lividomycin A from urine of dogs under acute renal failure was lower than that from normal.

Urinary excretion of lividomycin A was determined in rabbits given a single oral dose of 500 mg/kg with the results shown in Table 3. The total recovery of lividomycin A and kanamycin in the rabbits was 2 % and 3.3 % in 24-hour urine, respectively. This result indicates that lividomycin A, like kanamycin and gentamicin,[6] is not absorbed when given orally.

Fig. 1. Bioautograms of urine after intramuscular administration of lividomycin A

Urine : Dog, single dose 20 mg/kg
TLC adsorbent : Silica Gel G (Merck)
Solvent system : The upper layer of $CHCl_3$-MeOH-17% Ammonia (2 : 1 : 1)
A and C : Lividomycin A standard
B : Urine

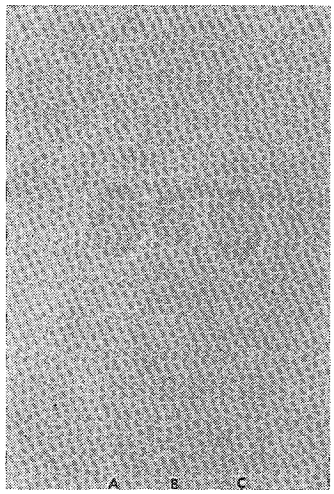

Table 7. Tissue distribution after intramuscular administration of a single dose (50 mg/kg) of lividomycin A in rats

Organs	Tissue levels (mcg/g or mcg/ml)				
	30 min.	1 hr.	3 hrs.	5 hrs.	12 hrs.
Brain	—	—	—	—	—
Liver	—	—	—	—	—
Kidney	62.7	29.3	34.3	22.9	19.3
Lung	10.6	3.8	2.4	1.4	—
Heart	3.1	—	—	—	—
Spleen	1.7	0.3	—	—	—
Muscle	6.1	1.9	—	0.8	—
Plasma	128.3	41.1	3.4	—	—

3. Metabolism of Lividomycin A

No active metabolite in urine from the animals that were administered lividomycin A was observed on bioautogram of thin-layer chromatography. The position of the inhibition zone was identical with that of authentic lividomycin A. The bioautogram is shown in Fig. 1. The result indicated that the active substance in urine remained unchanged in the form of lividomycin A. There was no evidence for the formation of a biologically active metabolite.

4. Biliary Excretion

Biliary excretion of lividomycin A was checked in dogs after intramuscular administration of a single dose of 50 mg/kg with the result shown in Table 6. The concentration in the bile reached a maximum of 8.7 mcg/ml 2 hours after administration and decreased rapidly thereafter.

5. Tissue Distribution in Rats

The results of tissue distribution studies in rats following intramuscular administration of a single dose of 50 mg/kg are summarized in Table 7. The peak of the tissue concentration was observed 30 minutes after administration with the concentration in blood (128.3 mcg/ml), followed by kidney (62.7 mcg/ml), lung (10.6 mcg/ml), muscle (6.1 mcg/ml), spleen (3.1 mcg/ml) and heart (1.7 mcg/ml) in order. Concentrations in all tissues except the kidney declined rapidly, while relatively high levels (19.3 mcg/ml) were still observed 12 hours after administration.

No lividomycin A was detected in the brain and liver tissues at any time. These results are similar to those reported for kanamycin[5] and gentamicin[6].

The high activity of lividomycin A against Gram-negative organisms[4], its rapid distribution throughout the body on intramuscular administration and high urinary concentrations that are achievable indicate therapeutic utility for the drug particularly in the treatment of urinary tract infections.

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