BIOLOGICAL STUDIES ON MIKAMYCIN. V

ABSORPTION AND EXCRETION OF TRITIATED MIKAMYCINS A AND B

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Mikamycins A & B containing mikamycin A as the main component and mikamycin B as the minor component were tritiated. The ^{3}H-mikamycins A & B were administered orally to mice in four doses of 0.1, 1.0, 10.0 mg/kg/day (for 10 days) and 50 mg/kg (single dose). The amount of ^{3}H-mikamycins A & B excreted into urine and feces was measured daily and totalled, and radioactivity remaining in the mice bodies was determined at intervals after administration ended. Thus, absorption, excretion and a material balance of mikamycins A & B were calculated. At every dose, more than 98 % of the radioactivity recovered was excreted in urine and feces within one to several days after administration ended.

Mikamycins A & B have a marked growth-promoting effect on broilers, chicks and poults when added in very low concentration to feed (1~2 mcg/g of feed). There are reports[1,2] that absorption of mikamycins A & B is relatively poor after oral administration and the concentration in blood is very low. Therefore, mikamycins A & B are not widely used for human therapy. For these reasons, mikamycins A & B are now being widely used for growth promotion of animals.

After oral administration of large doses of mikamycins, absorption and excretion have been measured by a differential assay[3] of mikamycin A and mikamycin B, and TANAKA *et al.*[4] reported their experimental results using tritiated mikamycin A, mikamycin B and mikamycins A & B. However, there is no comparable literature on the fate of such low level dose as are used to supplement animal feed. For easy and accurate measurement and establishment of material balances with small doses, ^{3}H-mikamycins A & B were used for the work described in this paper.

The standard dose by oral administration to mice was 0.1 mg of ^{3}H-mikamycins A & B per day per kg of mouse. This dose is almost the same as the daily intake of mikamycins A & B by broilers and chickens from commonly used supplemented feeds. In order to reduce experimental errors and keep practical conditions, administration was continued for 10 days. Tenfold (1 mg/kg/day, 10 days), hundredfold (10 mg/kg/day, 10 days) and five-hundredfold increases (50 mg/kg single dose) of the standard dose were examined for their effect on absorption and excretion. Not only the

absorption and excretion of ^{3}H-mikamycins A & B but also the material balances based on distribution of radioactivity are described in detail.

Materials and Methods

Preparation of Tritiated Mikamycins A & B: Two grams of finely ground mikamycins A & B, which were prepared in Kanegafuchi Chemical Iudustry Co., Ltd., were labelled with tritium gas at the National Institute of Health of Japan. In order to remove labile tritium, the material was dissolved in thirty volumes of methylisobutylketone and filtered. The precipitate obtained from the filtrate by adding ten volumes of *n*-hexane were dried *in vacuo*. After four repetitions of this treatment, the dried powder was suspended in one hundred volumes of distilled water, dried by lyophilization and the same treatment repeated. Tritiated mikamycins A & B were finally obtained as a powder with specific radioactivity of 42,300 dpm/mcg. Differential assay of this material showed that it contained 71.3 % of mikamycin A and 11.2 % of mikamycin B (mikamycins A & B 824.8 mcg/mg).

Administration of Tritiated Mikamycins A & B: The ddF male mice weighing 22±1 grams were administered orally a 5 % aqueous solution of dimethylsulfoxide or a suspension of tritiated mikamycins A & B in the following four doses:

1. 0.1 mg/kg/day (standard dose), ten days successively.
2. 1.0 mg/kg/day (tenfold dose), ten days successively.
3. 10.0 mg/kg/day (hundredfold dose), ten days successively.
4. 50.0 mg/kg/day (fivehundredfold dose), single dose.

Two mice on each dosage were sacrificed 1, 3, 7, 14, 21 and 28 days after administration ended. After a blood sample had been taken from under the left collarbone, the contents of gastrointestinal tract, and all organs, were separated and corresponding specimens from two mice were combined.

Extraction of Tritiated Mikamycins from Blood and Other Specimens: Blood samples were heparinized and centrifuged, then 0.2~0.5 ml of serum samples and 0.2~0.5 ml of hemolyzed blood cells were used for measurement of radioactivity. All separated and weighed tissues were homogenized in five volumes of 50 % methanolic buffer (Na_2HPO_4 4.2 g, KH_2PO_4 4.67 g, H_2O 1 liter, and methylalcohol 1 liter, pH 7.3), and 0.1~0.5 ml of the supernatant obtained by centrifugation was used for measurement of radioactivity.

The contents of the gastrointestinal tract were diluted to 10 ml with the methanolic buffer. After agitation and centrifugation, 0.5 ml of the supernatant was used for measurement of radioactivity. Feces and urine were collected daily after the start of administration, diluted with ten volumes of methanol and homogenized. After standing overnight at room temperature and centrifugation, 0.5 ml of the supernatant was used for measurement of radioactivity. The radioactivity excreted in urine and feces were added up daily and the totals were calculated.

Quantitative Determination of Tritiated Mikamycins A & B by Liquid Scintillation Counting: All samples were diluted with 7 ml of scintillator cocktail (PPO 6 g, naphthalene 100 g, and dioxane 1,000 mg) and their activities were measured with a Beckmann Scintillation Spectrometer.

Results

In the experiment with the standard dose (0.1 mg/kg/day, 10 days), the total radioactivity of ^{3}H-mikamycins A and B administered to two mice was 1.56×10^{5} dpm and the total amount was 36.8 mcg. Residual ^{3}H-mikamycins A and B in each organ one day after the end of administration and total excretion of ^{3}H-mikamycins A and B in urine and feces up to that time were calculated from the experimental results as shown in Table 1. The arranged data for various intervals are given in Table 2.

Similar calculations and arrangements of the data were carried out with all results of the other three experiments.

In Table 2, the ratio of total recovered ^{3}H-mikamycins A and B to the total administered (recovery %) and the ratio of total excreted ^{3}H-mikamycins A and B in urine and feces to the total recovered (excreted ratio %) were calculated.

Residual ^{3}H-mikamycins A and B concentrations were less than 0.2 mcg/g in lung, skin and peritoneum, and less than 0.1 mcg/g in other organs on one day after administration ended. After three days, concentrations of ^{3}H-mikamycins A and B in all organs were less than 0.02 mcg/g. Recovery of ^{3}H-mikamycins A and B was approximately 90 % at all intervals and the excreted ratios were 93.1 %, 99.3 % and 99.7 % at one, three and seven days after administration ended. Thus, it was ascertained from the experimental results that ^{3}H-mikamycins A and B were completely

Table 1. Distribution of ^{3}H-mikamycins A and B on 1 day after administration of the standard dose (0.1 mg/kg/day, 10 days) had ended.

	^{3}H/ml of the extract ($\times 10^{3}$ dpm)	Volume of the extract (ml)	Total ^{3}H of the extract ($\times 10$ dpm)	Total amount of mikamycins A and B (mcg)	Weight of the organ (mg)	Mikamycins A and B in organ (mcg/g)
Liver	0.29	15.6	4.52	0.11	3,566	0.03
Kidney	0.09	5.2	0.47	0.01	897	0.01
Spleen	0.12	1.8	0.22	0.01	307	0.02
Testis	0.62	2.1	1.30	0.03	356	0.09
Urinary bladder	0.08	0.4	0.03	0	57	0
Lung	1.27	2.2	2.79	0.07	381	0.17
Heart	0.12	1.4	0.17	0	230	0
Eye	0.54	0.5	0.27	0.01	83	0.08
Tongue	0.11	1.6	0.18	0	271	0
Brain	0.19	4.6	0.87	0.02	797	0.03
Diaphragm	0.22	0.7	0.15	0	129	0
Peritoneum	0.31	24.9	7.72	0.18	1,406	0.13
Omentum, fat and other tissues	0.53	19.6	10.39	0.25	4,732	0.05
Stomach	0.05	3.5	0.18	0	429	0
Content of the stomach	0.06	10.0	0.60	0.01	——	——
Small intestine	0.16	12.1	1.94	0.05	2,123	0.02
Contents of the small intestine	0.06	10.0	0.60	0.01	——	——
Large intestine	0.21	7.3	1.53	0.04	1,241	0.03
Contents of the large intestine	0.04	10.0	0.40	0.01	——	——
Skin	0.88	57.8	51.44	1.21	8,407	0.15
Muscle	0.06	70.9	4.25	0.10	12,512	0.01
Bone	0.36	30.6	11.02	0.26	5,069	0.05
Serum	1.28	0.8	1.02	0.02	0.8 ml	0.03(mcg/ml)
Erythrocyte	0.03	1.2	0.04	0	1.2 ml	0(mcg/ml)
Total in bodies			102.10	2.40		
Urine and feces			1,374.06	32.50		
Sum total			1,476.16	34.90		
Recovery			94.86%	94.84%		

The radioactivity of administered mikamycins A and B: 4.23×10^{7} dpm/mg.
Total radioactivity administered: 1.56×10^{6} dpm, that is 36.8 mcg of mikamycins A and B to two mice in ten days.

excreted in two or three days and there were no residual ^{3}H-mikamycins A and B in the body.

Experimental results from the tenfold dosage (1 mg/kg/day, 10 days) are shown in Table 3. Concentrations of ^{3}H-mikamycins A and B in the body after the final administration varied between 0.1~0.54 mcg/g at one day and 0.2 mcg/g or less at three days. Recovery of ^{3}H-mikamycyns A and B was 84~85 % at all intervals. Excreted ratio was 97.3 % at one day, 98.6 % at three days, and 98.9 % at seven days.

Absorption, excretion and material balances after hundredfold (10 mg/kg/day, 10 days) and fivehundredfold doses (50 mg/kg) are given in Table 4. The excreted ratio

Table 2. Absorption and excretion of ^{3}H-mikamycins A and B at intervals after administration of standard dose (0.1 mg/kg/day, 10 days) had ended.

	1 Day		3 Days		7 Days	
	Total amount of mikamycins A and B (mcg)	Mikamycins A and B in organ (mcg/g)	Total amount of mikamycins A and B (mcg)	Mikamycins A and B in organ (mcg/g)	Total amount of mikamycins A and B (mcg)	Mikamycins A and B in organ (mcg/g)
Liver	0.11	0.03	0.02	0.01	0	0
Kidney	0.01	0.01	0	0	0	0
Spleen	0.01	0.02	0	0	0	0
Testis	0.03	0.09	0	0	0	0
Urinary bladder	0	0	0	0	0	0
Lung	0.07	0.17	0	0	0	0
Heart	0	0	0	0	0	0
Eye	0.01	0.08	0	0	0	0
Tongue	0	0	0	0	0	0
Brain	0.02	0.03	0.01	0.01	0	0
Diaphragm	0	0	0	0	0	0
Peritoneum	0.18	0.13	0.01	0.01	0	0
Omentum, fat and other tissues	0.25	0.05	0.01	<0.01	0.07	0.01
Stomach	0	0	0	0	0	0
Contents of the stomach	0.01	——	0	——	0	——
Small intestine	0.05	0.02	0.03	0.02	0.01	<0.01
Contents of the small intestine	0.01	——	0	——	0	——
Large intestine	0.04	0.03	0.02	0.02	0	0
Content of the large intestine	0.01	——	0.01	——	0	0
Skin	1.21	0.15	0.03	<0.01	0	0
Muscle	0.10	0.01	0.04	<0.01	0	0
Bone	0.26	0.05	0.03	<0.01	0	0
Serum*	0.02	0.03	0.01	0.01	0.01	0.01
Erythrocyte*	0	0	0	0	0.01	0.01
Total in bodies	2.40		0.22		0.10	
Urine and feces	32.50		32.98		33.18	
Sum total	34.90		33.20		33.28	
Recovory**	94.84%		90.21%		90.43%	
Excreted ratio***	93.12%		99.34%		99.70%	

36.8 mcg of ^{3}H-mikamycins A and B was administered orally over a period of 10 days.
* In these cases weight of organ is replaced by volume in millilitres.
** Recovery means the ratio of total ^{3}H-mikamycins A and B recovered to the amount administered.
*** Excreted ratio (%) means the ratio of ^{3}H-mikamycins A and B in urine and feces to the total recovered sum total.

Table 3. Absorption and excretion of ^{3}H-mikamycins A and B at intervals after final administration

	1 Day		3 Days		7 Days	
	Total amount of mikamycins A and B (mcg)	Mikamycins A and B in organ (mcg/g)	Total amount of mikamycins A and B (mcg)	Mikamycins A and B in organ (mcg/g)	Total amount of mikamycins A and B (mcg)	Mikamycins A and B in organ (mcg/g)
Liver	1.43	0.54	0.25	0.10	0.13	0.05
Kidney	0.30	0.40	0.09	0.12	0.04	0.05
Spleen	0.05	0.22	0.04	0.09	0.01	0.02
Testis	0.05	0.15	0.03	0.09	0.01	0.04
Urinary bladder	0.01	0.15	0	0	0.01	0.09
Lung	0.07	0.18	0.08	0.23	0.10	0.26
Heart	0.03	0.15	0.02	0.08	0.01	0.06
Eye	0.01	0.16	0.01	0.08	0.01	0.10
Tongue	0.03	0.19	0.02	0.08	0.01	0.05
Brain	0.11	0.13	0.07	0.10	0.08	0.10
Diaphragm	0.01	0.13	0.01	0.09	0.01	0.07
Peritoneum	0.18	0.16	0.09	0.07	0.10	0.07
Omentum, fat and other tissue	0.43	0.10	0.14	0.05	0.17	0.04
Stomach	0.06	0.16	0.03	0.09	0.02	0.05
Content of the stomach	0.05	——	0.05	——	0.02	——
Small intestine	0.36	0.15	0.14	0.08	0.11	0.06
Contents of the small intestine	0.02	——	0.13	——	0.08	——
Large intestine	0.12	0.14	0.08	0.04	0.05	0.07
Content of the large intestine	0.50	——	0.07	——	0.05	——
Skin	0.62	0.10	0.51	0.07	0.54	0.06
Muscle	1.33	0.13	0.88	0.08	0.67	0.05
Bone	0.65	0.10	0.49	0.09	0.26	0.04
Serum	0.17	0.24	0.11	0.14	0.17	0.21
Erythrocyte*	0.15	0.14	0.09	0.11	0.13	0.12
Total in bodies	6.74		3.43		2.79	
Urine and feces	242.59		244.62		246.30	
Sum total	249.33		248.05		249.09	
Recovery**	84.81%		84.37%		84.72%	
Excreted ratio***	97.30%		98.62%		98.89%	

294.0 mcg of ^{3}H-mikamycins A and B was administered orally over a period of 10 days.
* In these cases weight is replaced by volume milliliters.
** Recovery means the ratio of total ^{3}H-mikamycins A and B recovered to the total administered.
*** Excreted ratio (%) means the ratio of ^{3}H-mikamycins A and B in urine and feces to the total recovered.

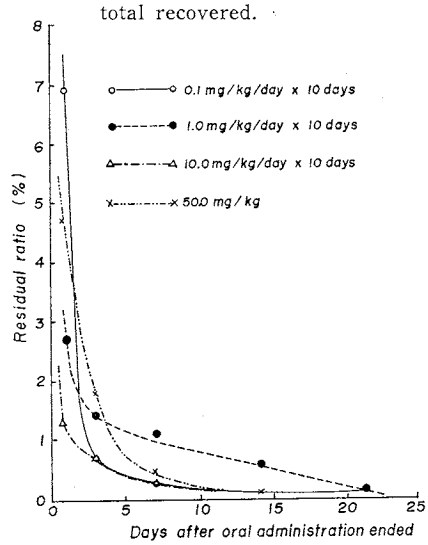
Table 4. Absorption, excretion and balance of ^{3}H-mikamycins A and B at the high dose level.

Days after administration ended		1	3	7	14	21
Oral administration (10 mg/kg/day ×10 days)	Total in bodies (mcg)	39.8	18.9	8.8	2.7	2.3
	Total in urine and feces (mcg)	2,735.3	2,741.8	2,747.4	2,750.4	2,756.9
	Sum total (mcg)	2,775.1	2,760.7	2,756.2	2,753.1	2,759.2
	Recovery (%)	86.7	86.3	86.2	86.1	86.2
	Excreted ratio (%)	98.7	99.3	99.7	99.9	99.9
Oral administration (50 mg/kg)	Total in bodies (mcg)	64.1	24.0	5.3	2.5	1.5
	Total in urine and feces (mcg)	1,295.1	1,310.5	1,319.4	1,326.5	1,329.7
	Sum total (mcg)	1,359.2	1,334.5	1,324.7	1,329.0	1,331.2
	Recovery (%)	78.8	77.3	76.8	77.0	77.1
	Excreted ratio (%)	95.3	98.2	99.6	99.9	99.9

of the standard dose (1.0 mg/kg/day, 10 days).

14 Days		21 Days	
Total amount of mikamycins A and B (mcg)	Mikamycins A and B in organ (mcg/g)	Total amount of mikamycins A and B (mcg)	Mikamycins A and B in organ (mcg/g)
0.07	0.03	0	0
0.01	0.02	0	0
0.01	0.01	0	0
0.01	0.03	0	0
0.01	0.19	0	0
0.01	0.02	0.03	0.04
0	0	0	0
0	0	0	0
0.02	0.11	0	0
0.01	0.02	0	0
0	0	0	0
0.02	0.02	0	0
0.45	0.13	0.02	<0.01
0.03	0.07	0	0
0.02	——	0	——
0.04	0.02	0.01	<0.01
0.02	——	0	——
0.37	0.37	0	0
0.02	——	0	——
0.09	0.01	0.07	0.04
0.15	0.02	0.02	<0.01
0.08	0.02	0.04	0.01
0.04	0.03	0.02	0.02
0.01	0.01	0	0
1.49		0.21	
246.73		246.90	
248.22		247.11	
84.42%		84.05%	
99.40%		99.92%	

Fig. 1. Residual ratio of ^{3}H-mikamycins A and B in the body to the total recovered.



at the hundredfold dose was 98.7 % at one day and 99.3 % at three days after administration ended. The ratios after 50 mg/kg oral administration were 95.3 % at one day and 98.2 % at three days. With these two high level doses, the concentrations of ^{3}H-mikamycins A and B in all organs or tissues were less than 0.1 mcg/g after fourteen days.

The residual ratios of ^{3}H-mikamycins A and B in the body to the total recovery at the four different dose levels are shown in Fig. 1. From this it is clear that excretion of ^{3}H-mikamycins A and B are rapid and residual ratio is very low, regardless of the changes in dose levels.

Comment

The possibility of prolonged existence of mikamycins A and B or its degradation products in organs and tissues is considered unlikely by the experimental results obtained when a standard low level dose of ^{3}H-mikamycins A and B was administered to mice. This information is important in evaluating the use of mikamycins A and B as a supplement in animal feeds.

Material balances of ^{3}H-mikamycins A and B were investigated for four different administrations. Regardless of whether low or high level doses were used, there was

no tendency for ^{3}H-mikamycins A and B to accumulate in specific tissues or organs, and most of ^{3}H-mikamycins A and B were rapidly excreted in urine and feces.

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