STUDIES ON ANTITUMOR ACTIVITY OF PRUMYCIN
II. STUDIES ON DISTRIBUTION AND EXCRETION OF PRUMYCIN*

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Tissue distribution, excretion and metabolism of prumycin in normal mice and rats were studied by microbiological assay. Following the injection of prumycin into mice, high activity was detected and continued for 24 hours in the kidney, and the activity was also high in the skin, uterus, bone, liver, lung and stomach in this order. But concentration in the brain, heart, spleen and testis were too low to detect even 5 minutes after the injection. Prumycin was not inactivated by a variety of tissue homogenates *in vitro*. Therefore, inability to detect activity of prumycin in the spleen and testis appears to result from poor distribution rather than inactivation by these organs. About 70% of injected prumycin was excreted into rat urine in 24 hours but it was not detectable in feces. When prumycin was injected intravenously into dogs at the dose over 10 mg/kg, vomiting was observed in all animals, and LD_{50} was about 50 mg/kg.

It was found that prumycin, an antifungal antibiotic discovered by HATA *et al.*[1,2], was effective against several experimental tumors, specially against mammary adenocarcinoma as previously reported[4]. On the other hand, prumycin produced alopecia in mice, whereas it did not show the significant bone marrow toxicity[4]. These phenomena suggest that prumycin may be distributed in the skin to a higher degree than bone marrow. The present study was designed to investigate the tissue distribution, metabolism and excretion of prumycin in normal mice and rats. Toxic dose, side effects and serum level of prumycin in dog are also reported in this paper.

Materials and Method

Animals

Male and female *ddY* mice weighing about 20 g, and male Donryu rats, about 150 g body weight were purchased from Shizuoka Agriculture Cooperative Association for Laboratory Animals and Nihon Rat Co. Male and female dogs were obtained from Laboratory Research Enterprises, Inc.

Chemicals

Prumycin and mitomycin C were obtained as previously reported[4].

Distribution of prumycin in mice

Prumycin was intravenously injected into normal mice at the dose of 144 mg/kg (LD_{50}), and 5, 10, 30, 60 and 120 minutes after injection four mice were bled and sacrificed. Each tissue was quickly obtained, rinsed, weighed and homogenized with phosphate buffered saline. Concentration of prumycin in tissue homogenate was determined by the cylinder-plate method using *Micrococcus lutea* PCI 1001 as the test organism.

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Inactivation of prumycin and mitomycin C by mouse tissue homogenates

Various tissues were homogenized with phosphate buffered saline at the concentration of 20%. And same volume of prumycin or mitomycin C solution (100 mcg/ml) was added and the mixture was incubated at 37°C for 60 minutes. Residual antimicrobial activity was determined by the cylinder-plate method.

Excretion of prumycin in mice and rats

Prumycin was intravenously injected into normal *ddY* mice and Donryu rats at the dose of 72 mg/kg and 30 mg/kg respectively. Then urine and feces excreted by 24 hours after injection, and also within one more 24 hours after were collected. Feces were weighed and extracted with water. Concentration of prumycin in urine and feces extract was determined by the cylinder-plate method.

Bioautogram of antimicrobially active metabolite of prumycin after incubation with tissue homogenate and in urine

Samples were spotted on thin-layer chromatography plate (silica gel 60, Merck) and developed with the butanol - acetic acid - water system (3: 1: 2) as reported by HATA *et al.*[1]

Results

Tissue Distribution of Prumycin

Prumycin was intravenously injected into mice at the dose of 144 mg/kg. As shown in Table 1, serum level of prumycin at 5 minutes after the injection was 210 mcg/ml and declined steadily thereafter. High concentration of prumycin was measured in the kidney, skin, uterus, bone, liver, lung and stomach. On the other hand the antibiotic was not detected in brain, heart, spleen and testis even 5 minutes after the injection. Concentration of prumycin was under the limit of detection at 2 hours after the injection in all tissues except kidney, in which prumycin could be detected even 24 hours after injection.

Inactivation of Prumycin and Mitomycin C by Various Mouse Tissue Homogenates

Table 2 shows the result of residual antimicrobial activity of prumycin and mitomycin C after the incubation with various tissue homogenates. Prumycin was not inactivated by any tissue homogenate

Table 1. Tissue distribution of prumycin.

Tissues	Time after the administration							
	5 min.	10 min.	30 min.	1 hr.	2 hrs.	4 hrs.	24 hrs.	72 hrs.
Serum	210[a]	144	60	13	—	—	—	—
Kidney	368[b]	203	110	60	53	42	11	—
Skin	104	53	28	<17				
Uterus	94	52	<47					
Bone	84	51	40					
Liver	81	74	41	<19				
Lung	78	40	<40					
Stomach	64	25	<24					
Small intestine	36	22	<18					
Peritoneum	35	28	<22					
Brain	<24							
Heart	<30							
Spleen	<27							
Testis	<27							

Prumycin was administered intravenously into normal mice at the dose of 144 mg/kg. a) mcg/ml b) mcg/g

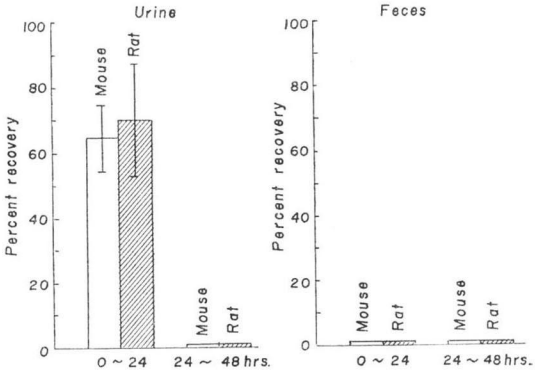
Table 2. Inactivation of prumycin and mitomycin C by various mouse tissue homogenate.

Tissue homogenate	Residual antibacterial activity (%)	
	Prumycin	Mitomycin C
Serum	100	98
Skin	106	
Liver	102	11
Spleen	96	
Kidney	98	
Lung	102	
Uterus	106	
Brain	98	
Peritoneum	98	
Stomach	94	
S. intestine	106	
Testis	102	
Sarcoma 180 ascites cells	97	32

Prumycin and mitomycin C solution (100 mcg/ml) were mixed with same volume of 20% tissue homogenate, and incubated at 37°C for 60 minutes.

Fig. 1. Excretion of prumycin into urine and feces in mice and rats.

Prumycin was intravenously injected into mice and rats at the dose of 72 mg/kg and 30 mg/kg, respectively.



and sarcoma 180 cells at all, whereas mitomycin C was quickly inactivated by the same liver homogenate and sarcoma 180 cells.

Excretion of Prumycin

Prumycin recovered in urine and feces of normal mice and rats was expressed in percent of amount injected and is shown in Fig. 1. After the intravenous injection of 72 mg/kg into mice and 30 mg/kg into rats, about 65% and 70% of injected prumycin were recovered in the first 24-hour urine samples collected from these animals. But additional excretion of the antibiotic in the second 24-hour urine samples was trace in mice and rats. Detectable amount of prumycin could not be found in feces 24 hours after the injection in both animals.

Bioautogram of Active Metabolite of Prumycin in the Mixture with Tissue Homogenate and in Urine

An antimicrobial active substance in the mixture of prumycin and tissue homogenate, and in urine of rat was identified as prumycin itself by the same Rf value (0.23) on the thin-layer chromatography plate.

Toxicity and Side Effects of Prumycin in Dogs

Toxicity and side effects of prumycin in dogs after intravenous injection at the dosage from 20 mg/kg to 100 mg/kg are shown in Table 3. One dog each received 100 mg/kg and 50 mg/kg of prumycin died within 48 hours. But all dogs received the dosage from 10 mg/kg to 30 mg/kg survived and showed no change of white blood cell counts and BUN, though vomiting was observed about 10 minutes after the intravenous injection in all cases. And LD_{50} seemed to be around 50 mg/kg.

Serum Level of Prumycin in Dogs

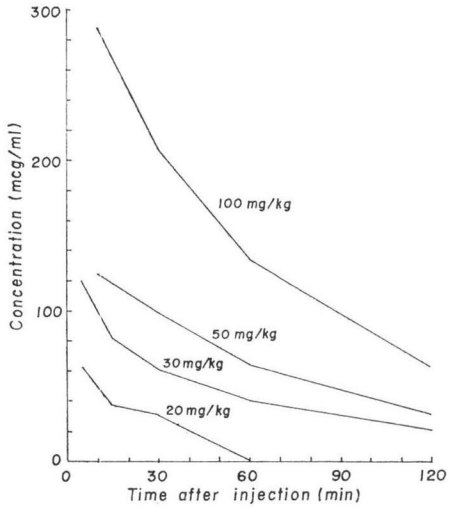
Fig. 2 shows time course of serum level of prumycin after the intravenous injection into dogs. Serum level in dogs was almost parallel with the dosage administered and higher than that in mice as seen in Table 1.

Table 3. Toxicity and side effects of prumycin in dog.

Dose mg/kg	Items of examination	Time after the administration				
		Time 0	(Day 0)	Day 3	Day 5	Day 14
100	Mortality		1/1			
	Symptoms		Vomiting			
50	Mortality		1/2	1/2	1/2	1/2
	Symptoms		Vomiting			
30	Mortality		0/1	0/1	0/1	0/1
	Symptoms		Vomiting			(no alopecia)
	Body wt. (kg)	11		10	11	11
	WBC (/mm^3)	11,700		10,900	11,700	11,500
	BUN (mg/dl)	14.6		16.3	14.0	15.0
20	Mortality		0/2	0/2	0/2	0/2
	Symptoms		Vomiting			(no alopecia)

Prumycin was injected intravenously into beagle dogs.

Fig. 2. Serum level of prumycin in dogs after intravenous injection.



Discussion

In a previous paper we reported that prumycin had antitumor activity against some experimental tumors without depression of the white blood cell counts, though it caused severe alopecia in mice[4]. The present study was designed to investigate the tissue distribution, metabolism and excretion of this antibiotic. Although prumycin is inactive against most bacteria and yeasts, it has been shown to be active against *Micrococcus lutea* PCI 1001 as well as against phytopathogenic fungi such as *Sclerotinia cinerea*[1,2]. Therefore, *Micrococcus lutea* PCI 1001 was used as the test organism for microbiological assay of prumycin.

The concentration of prumycin in kidney was high and remained for 24 hours after the injection into mice of the 144 mg/kg dose, approximately 70% of the active drug was recovered in rat urine, without the identificating active metabolites. Prumycin can be classified as one of aminoglycoside antibiotics from its structure, 4-D-alanyl-2,4-diamino-2,4-dideoxy-L-arabinose, determined by ŌMURA *et al.*[3] High concentration in kidney, high recovery in urine and no metabolites seem to be characteristics of the distribution, excretion and metabolism of aminoglycoside antibiotics[5~8]. In a previous experiment[4], histological change was not observed in the kidney after a single injection of prumycin (75 mg/kg) into mice. In the present experiment BUN (blood urea nitrogen) was not increased in dogs by a single intravenous injection of prumycin at a dose equivalents to its LD_{50}. LUFT and KLEIT[6] measured the half-life of several aminoglycosides in the kidney after a single subcutaneous injection and showed that streptomycin had a shorter half-life (4.6 hours) than gentamicin (109 hours), tobramycin and kanamycin. The degree of accumulation of prumycin in the kidney seems to be considerably small compared to gentamicin on report by LUFT and KLEIT, when it was still detected in the rat kidney 11 days after a single subcutaneous injection of 10 mg/kg. After the injection

of prumycin, high activity was also detected in skin, uterus, bone, liver, lung and stomach in that order, whereas brain, spleen, heart and testis showed undetectable activity. Alopecia which was observed in mice after the injection of prumycin might be related to the high concentration of this antibiotic in skin. It was also detected in bone at high concentration, though we described that prumycin did not cause bone marrow toxicity.[4] The difference of the concentration of prumycin in various organs probably is not due to the distribution of inactivating enzyme because prumycin was not inactivated by any tissue homogenates and no active metabolites could be found in urine.

It is interest to note that changes in WBC (white blood cells) and BUN and alopecia could not be observed in dogs at the dosage equivalent to its LD_{50}. However vomiting was caused by prumycin at a dose above 10 mg/kg. But some aminoglycoside antibiotic was demonstrated to locate at cartilaginous tissues rather than bone marrow by autoradiography[9].

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