TISSUE PHARMACOKINETICS AND INHIBITION OF DNA SYNTHESIS IN MICE TREATED WITH SPORAMYCIN

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Examination of blood and organ concentrations of sporamycin in normal mice showed a rapid decrease of sporamycin from peripheral blood and a high level of sporamycin in urine 10 minutes after intravenous injection of the antibiotic. At the same time, the highest level was found in the kidneys and low levels were found in the lungs and spleen. When sporamycin was added to mouse organ homogenate at 37°C, remarkable inactivation of sporamycin by the homogenate of the liver, kidney, testis, *etc.*, was noted but this inactivation was slight by tumor homogenate. Sporamycin inhibited the tritiated thymidine incorporation into DNA of normal tissues, but a different pattern of inhibition and recovery on the incorporation of ^{3}H-TdR into DNA was observed among mouse organs. It was noted that the antibiotic may damage normal tissues in spite of a rapid excretion and inactivation of sporamycin in mice, but this damage was recovered rapidly within 1~2 days after the treatment.

Sporamycin is a new polypeptide antitumor antibiotic produced by *Streptosporangium pseudovulgare* strain No. PO-357.[1,2] This antibiotic has recently been found to be effective in several murine tumors *in vivo* and several human tumor cells *in vitro*.[3] We found that sporamycin strongly inhibited the incorporation of tritiated thymidine (^{3}H-TdR) into DNA of HeLa cells *in vitro*, in spite of very slight inhibition of ^{3}H-uridine incorporation into RNA and ^{3}H-leucine incorporation into protein.

The purpose of this study was to investigate the distribution of sporamycin in mice, inactivation of the antibiotic by mouse organs and tumor homogenates, and inhibition of the uptake of ^{3}H-TdR into DNA of mouse organs after a single injection of the antibiotic into mice.

Materials and Methods

Animals

Adult male dd and BDF_1 mice weighing 22~24 g were used. For the investigation on the blood level and organ distribution, mice were given a single intravenous injection of sporamycin.

Blood Level

Blood was drawn periodically from caudal vein of 8 mice at each time, and the antibacterial activity of sporamycin in blood was measured by the paper disk method on an agar plate using *Staphylococcus aureus* as the test organism. To make the standard curve for the estimation of blood level, sporamycin was diluted with blood of the mouse.

Preparation of Samples for Organ Distribution

Normal dd mice were given 50 or 200 mg/kg of sporamycin and were sacrificed at certain intervals after the injection. The organs pooled from groups of 6 mice were chilled rapidly, and immediately made into a 20% homogenate in sterilized water, with a homogenizer in ice-cold water. Antimicrobial activity of the homogenate was measured by the paper disk method on an agar plate using *Staphylococcus aureus* as the test organism. To make the standard curve for the estimation of organ level, sporamycin was diluted with ice-cold 20% homogenate of each organ from the mouse.

Inactivation of Sporamycin by Organ Homogenate

Various organs of mice were prepared into a 20% homogenate, a quantity of sporamycin was added to each homogenate to make the final concentration of the antibiotic to 1 mg/ml, and the mixtures were incubated for 1 or 3 hours at 37°C. Antimicrobial activity was measured by the same method as described above, then the residual activity was calculated as 100% at 0 hour.

Thymidine Incorporation into DNA

Normal mice were given intravenous injections of thymidine[6-^{3}H] (^{3}H-TdR), 10 μCi/10 g body weight, at 60 minutes before sacrifice. Three of the mice in one group were killed by cervical dislocation. DNA was extracted by a slight modification of the ORLOV and ORLOVA procedure.[4] About 150~200 mg of tissue sample from each mouse was heated in 3 ml of 1 N NaOH in a boiling water bath for 5 minutes and then chilled in ice-cold water. This mixture was added with 1.5 ml of a solution of 20% acetic acid saturated with NaCl. After standing in an ice bath for 2 hours, the samples were centrifuged and 3.5 ml of the supernatant was mixed with 7 ml of cold 95% ethanol. After standing in an ice bath over night, the precipitate formed in the ethanol solution was collected by centrifugation, and washed twice with cold 5% trichloroacetic acid, twice with cold 95% ethanol, and twice with 2: 1 solution of ethanol - ether at 50°C for 10 minutes.

DNA Measurement

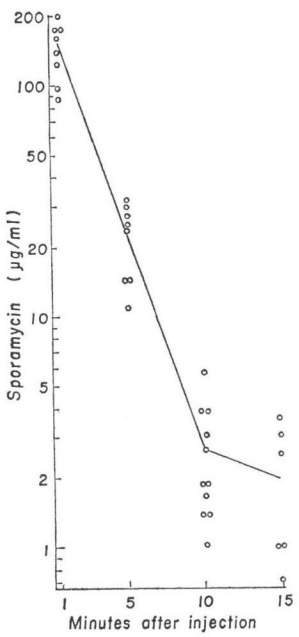
The above precipitate was hydrolyzed in 2 ml of 0.5% $HClO_4$ for 15 minutes at 70°C. The hydrolyzate was used for the determination of DNA by the diphenylamine method and for liquid scintillation counting. A Packard Tri-Carb liquid scintillation spectrometer (Model 3357) was used for the counting, and counts were corrected for quenching. The specific activity of DNA was expressed as dpm/μg DNA. The experiment for ^{3}H-TdR incorporation into DNA was duplicated and mean value was calculated from each value of 6 mice.

Results

Blood Level of Sporamycin

It was found from the antibacterial activity against *Staphylococcus aureus* that the blood level of sporamycin decreased rapidly, as shown in Fig. 1. At 1 minute after injection of 50 mg/kg of sporamycin, the blood level was approximately 160 μg/ml, but only 2.6 μg/ml was found after 10 minutes, and a very slight antibacterial activity was found at 30 minutes.

Fig. 1. Blood level of sporamycin.
Mice were given 50 mg/kg of sporamycin intravenously. Open circles indicate blood level of each mouse.



Organ Level of Sporamycin

The results shown in Table 1 represent the mean values of 8~10 mice for each value. At 10 minutes after injection of the antibiotic, the kidneys had the highest antibacterial activity, followed by the lung and spleen, to a slight degree. No activity was found in the liver, brain, testis, pancreas, or small intestine, even at a dose of 200 mg/kg. A very high activity was found in the urine already 10 minutes after the injection, but the activity was not observed in the bile. At 30 minutes, concentration of sporamycin in the kidneys and lungs decreased rapidly, but a high activity was still observed in the urine.

Inactivation of Sporamycin by Homogenates of Organs and Tumors

As shown in Fig. 2, the antibacterial activity of sporamycin was

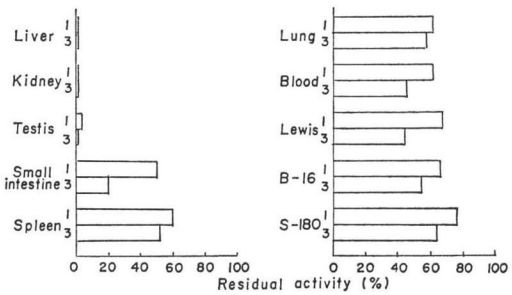
Table 1. Organ concentration of sporamycin in mice

Dose (mg/kg)	Minutes after injection	μg/g or ml wet tissue			
		Kidney	Lung	Spleen	Urine*
50	10	27.5	1.6	—	5,700
	30	9.2	0.1	—	3,400
200	10	123.0	25.0	2.0	>10,000
	30	27.3	2.2	1.9	5,630

* Urine was collected directly on a paper disk when animals were sacrificed.

completely or almost completely lost when sporamycin was incubated with the homogenate of liver, kidney, or testis at 37°C for 1 or 3 hours. The antibiotic was moderately inactivated by the homogenate of B-16 melanoma, sarcoma-180, or LEWIS lung carcinoma at 37°C.

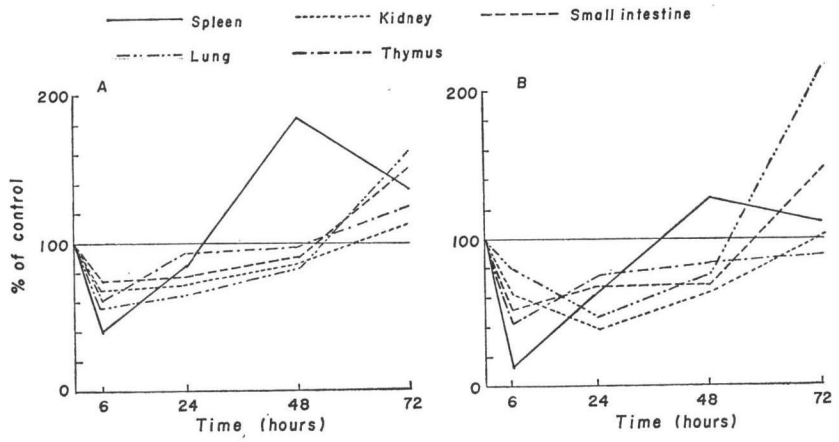
Fig. 2. Inactivation of sporamycin by 20% homogenate of mouse organs and of tumors.
Mixtures of homogenate and sporamycin were incubated at 37°C. Numbers on the ordinate indicate incubation time (hours).



Effect of Sporamycin on ^{3}H-TdR Incorporation into DNA of the Organs in the Normal Mouse

Changes in DNA synthesis in normal mouse organs after single doses of sporamycin at 5 and 2.5 mg/kg intravenously are shown in Fig. 3. In each of the 5 organs at each of the two doses, there is a marked inhibition of ^{3}H-TdR incorporation into DNA. This inhibition is evident at 6 hours and maximal at 6~24 hours. The magnitude and persistence of the inhibition of DNA synthesis seem to depend on the dose. This inhibition was followed by a rapid recovery of ^{3}H-TdR incorporation into DNA and by an overshoot phase of the incorporation within 1~3 days, followed in turn by a return to the pretreat-

Fig. 3. Effect of sporamycin on the incorporation of ^{3}H-thymidine into DNA of spleen, kidneys, small intestine, lung and thymus.
(A) Sporamycin, 2.5 mg/kg iv. (B) Sporamycin, 5.0 mg/kg iv.



ment levels. With each of the two doses tested, ^{3}H-TdR incorporation into the spleen was more profoundly suppressed but recovered more rapidly than the other tissues.

Discussion

After intravenous injection of a single dose of sporamycin to normal mice, the antibiotic disappeared rapidly from the peripheral blood, and it appeared in the urine at 10 minutes later. Examination of the organ distribution showed that the greatest concentration accumulated in the kidneys. It appeared therefore, that a rapid decrease of blood concentration is mainly due to the rapid excretion of the free antibiotic into urine. These findings were similar to those for another macromolecular antitumor antibiotic, neocarzinostatin.[5,6,7]

We reported previously that sporamycin was bound rapidly to HeLa cells *in vitro*, thereby inhibiting cell growth.[3] Hence, though a rapid excretion of sporamycin was observed, a sufficient amount of sporamycin to inhibit the cell growth is expected to bind rapidly to the cells *in vivo*, because a remarkable suppression of ^{3}H-TdR incorporation into DNA was observed in the cells. It has been reported that neocarzinostatin was considerably inactivated by the homogenate of liver, kidney, testis, *etc., in vitro*.[5] Sporamycin was also inactivated by the homogenate of liver, kidney, and testis but not so markedly by that of the spleen. A slight antibacterial activity was observed in the spleen from the examination of organ concentration, and the uptake of ^{3}H-TdR into DNA of spleen cells was strongly inhibited. In addition, in spite of the greater accumulation of the antibiotic in the kidney, inhibition of ^{3}H-TdR into DNA of kidney cells was not so different from that of the spleen. PHILIPS *et al.*[8] and TAYLOR *et al.*[9] have reported the discrepancies between the amount of a drug in the tissue and extent of the inhibition of DNA synthesis. In the case of sporamycin, several reasons will be considered, but it seems that the inhibitory effect of sporamycin on the incorporation rate was correlated mainly with susceptibility of the tissues to cytotoxicity rather than to organ concentrations. In normal organs, there was a prompt initial depression of ^{3}H-TdR incorporation into DNA at 6 hours after the administration of 5 mg/kg of sporamycin which demonstrated a remarkable antitumor effect, according to a previous paper.[2,3] This depression was followed by a rapid recovery of DNA synthesis within 1~2 days. Thus, sporamycin may damage normal organs but these normal organs appear to recover rapidly.

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