Notes

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Toxicological Approaches to Streptothricin Antibiotics. II. The Developmental Mechanism of Delayed Toxicity in Mice and Rats[1]

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In order to investigate the mode of the delayed toxicity of streptothricin antibiotics, ^{14}C-glycyl-racemomycin-A and racemomycin-D were each administered intravenously to mice and rats in a seuviving dose. As to the distribution of ^{14}C-glycyl-racemomycin-A in the organs of mice, studies confirmed us that it was distributed in kidney in a high concentration in comparison with the distribution in the other organs. Therefore, pathohistological reviews were carried out on the kidney which was whitened macroscopically. As a result, severe toxicity wide-spread in the cortex renis was observed in the kidney. From these results, it was recogenized that streptothricin antibiotics produce nephrotoxicity.

As to the developmental mechanism of the delayed toxicity, the authors presented the assumption that after the administered antibiotic us, *in vivo*, transformed into acid, lactam ring of which is broken from examination of radiochromatogram of metabolite in urine, and that the acute toxicity of that acid is responsible for the delayed toxicity.

Keywords——delayed toxicity; racemomycin-A; ^{14}C-labeled glycyl-racemomycin-A; pathohistological review; renal cortex; renal toxicity; distribution of ^{14}C-labeled glycyl-racemomycin-A

The antibacterial action of streptothricin antibiotics is of broad spectrum. The delayed toxicity, however, is so severe as to prevent the antibiotics from being put to practical use. Nevertheless, the antibiotics have been proved to be effective on some kinds of viruses,[3] while the toxicological approaches have been promoted with the view of reducing the delayed

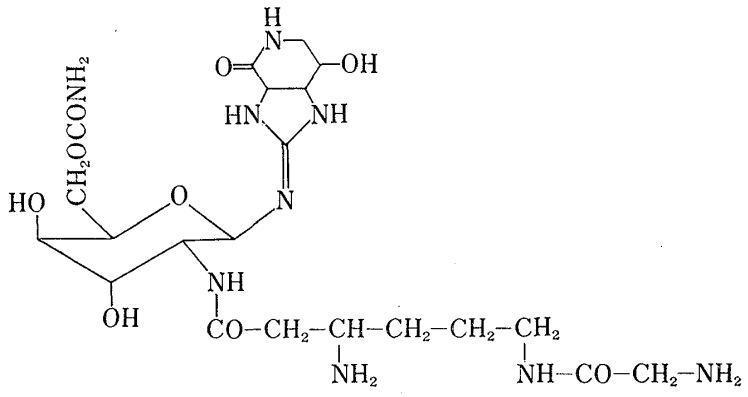

Fig. 1. Chemical Structure of Glycylracemomycin-A

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toxicity by chemical modification.[4] In preceding reports, the authors studied the pharmacokinetics of racemomycin-A and-D in the group of streptothricin antibiotics in terms of antibacterial activities for the purpose of throwing some light on the developmental mechanism of the delayed toxicity. It was reported that the antibiotics were distributed in the kidney with high concentration and that racemomycin-A might be inactivated and excreted into urine, judging from the rate of recovery from the urine during the first 24 hours.[5] The present report describes the findings on the pharmacokinetics of ^{14}C-labeled glycyl-racemomycin-A (Fig. 1) and on the developmental mechanism of the delayed toxicity of racemomycin-D from the clinico-chemical and patho-histological viewpoints.

Materials and Methods

Animals——Male ddY-strain mice (each weighed 28 to 29 g) were used in the experiment of the pharmacokinetics of ^{14}C labeled glycyl-racemomycin-A. For the patho-histological and the clinico-chemical(plasma GOT and GPT) examinations of racemomycin-D, male Wistar-strain rats (each weighed 200 to 220 g) were used.

Antibiotics——^{14}C-labeled racemomycin-A obtained through biosynthesis, was weak in radioactivity, but ^{14}C labeled glycyl-racemomycin-A, which was chemically synthesized from racemomycin-A and glycine-U-^{14}C, showed the radioactivity of 42271 dpm/mg. Accordingly, the latter was employed in place of the former. Racemomycin-D[6] was obtained from defined culture broth of *Streptomyces lavendulae* OP-2 strain.[7] The antibiotics were dissolved in sterile distilled water, and injected into the tail veins of mice and rats. Doses were 200 mg/kg in ^{14}C-labeled glycyl-racemomycin-A,[5] and 40 mg/kg in racemomycin-D,[5] for the laboratory test and the patho-histological examination.

Radioactivity Detection——After intravenous injection of ^{14}C-labeled glycyl-racemomycin-A, mice were sacrificed at the given intervals. The excised organs were washed in saline, and weight was determined. In the cases of blood and urine, they were made to sink into the filter papers previously weighed, and then the papers were again weighed, and the weight of blood and urine was calculated. To measure specific radioactivity, the papers and each organs were burned by means of Sample Oxizider (Packerd 306).

Detection of the Metabolites in Urine——After intra venous injection of ^{14}C-labeled glycyl-racemomycin-A, mice were maintained in cages for 24 hours, to collect urine. The definite quantity of the urine was put to spot tests on the filter paper (Toyo-roshi No. 51) for paper chromatography by the use of the solvent system of *n*-BuOH–pyridine–AcOH–H_2O-*tert*-BuOH (15: 10: 3: 12: 4). Studies were carried out on the radiochromatogram and paper chromatogram after development with ninhydrin.

Pathohistology——Although the weight of the liver and spleen decreased remarkably after the administration of racemomycin-D, that of kidney did not decrease as in the preceding report.[5] However, the kidney was pathohistologically examined, since it was macroscopically observed to be whitened Racemomycin-D (40 mg/kg) was administered to rats, and the rats were sacrificed four days later. The three kinds of organs (liver, spleen and kidney) were put to hematoxylin eosin staining for pathohistological study.

Determination of Plasma GOT and GPT——The rats were distributed in groups of five rats, and racemomycin-D (40 mg kg) was injected into the tail veins. The collection of blood was carried out, one day, three days, and five days after the injection. Blood samples were obtained from the heart of the rats anesthetized with pentobarbital and treated with heparin. The blood samples were centrifuged at 3000 rpm for 10 to 15 minutes to yield plasma GOT and GPT values were determined by the method of hydrazone colorimetric analysis.[8] Plasma trans-amidase reagents (Fujimoto Pharma. Mfg. Co., Ltd) were employed for the determination of GOT and GPT. The calibration curve of GOT and GPT was produced by the use of 2 ml pyruvic acid standard solution.

Results

Pathohistological Examination

After the administration of racemomycin-D, the kidney, liver and spleen of the rat was histologically examined four days later. The atrophy of the liver and spleen was observed

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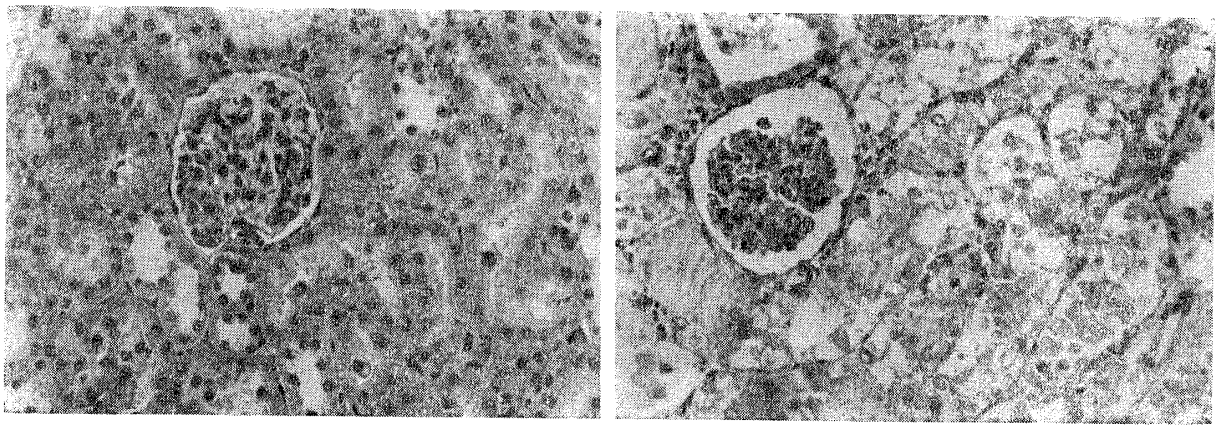

a b

Fig. 2a, b. Photograph of Renal Cortex of Normal Rat and Racemomycin-D Administered Rat

a: renal cortex of normal rat (hematoxylin eosin staining, ×400).
b: renal cortex of racemomycin-D administered rat (hematoxylin eosin staining, ×400).

TABLE I. The Values of Plasma GOT and GPT after Racemomycin-D Administration

	Control days			40 mg/kg (*i.v.*) days		
	1	3	5	1	3	5
GOT[a)]	23.7±6.3	24.0±3.3	17.3±3.1	24.5±7.6	14.8±3.1	11.3±3.0
GPT[a)]	24.5±7.1	13.8±3.1	11.1±2.9	24.5±7.1	14.8±4.5	10.6±2.8

Animals: Wistar strain (♂) rat body weight 200—220 g 5/group.
Route: intravenous injection.
Dose: 40 mg/kg *a*) Feitman Rrankel unit.
Each value represents the mean±SE of 10 experiments.

macroscopically, but no pathological change was observed. As to the kidney, the wide-spread degeneration of the cortex reins was observed as shown in Fig. 2b, in comparison with the cortex reins (Fig. 2a) of normal rats. The pathohistological changes in the kidney caused by antibiotics were strong necrosis of the epithelium of proximal tubule, and weak necrosis of the distal tubules in renal cortex.

Changes of Plasma GOT and GPT Values

Racemomycin-D was administered to rats, and the blood samples were collected, one day, three days, five days later, to measure the values of GOT and GPT (Table I).

After the administration of racemomycin-D, the values of plasma GOT and GPT were not great difference in comparison with these values (Reitman Frankel unit) of control group.

These findings led to the judgement that the values of GOT and GPT after the administration were in the range of normal values.

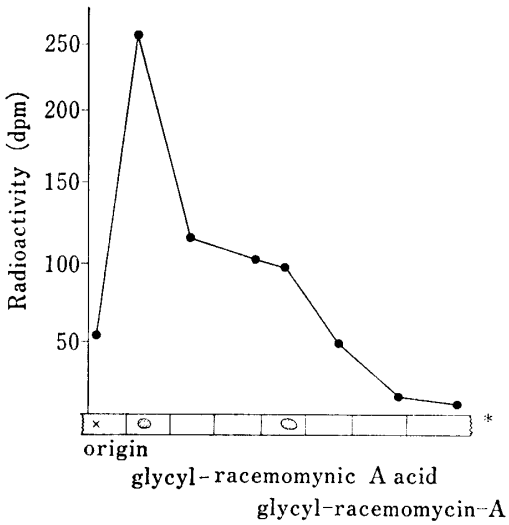

Fig. 3. Radioactivity Scanning of Urinary Components from ^{14}C-Glycyl-Racemomycin-A on Paper Chromatogram

* Toyo-Roshi No. 51 ascending method.
Solvent system: *n*-BuOH-pyridine-AcOH-H_2O-*tert*-BuOH (15: 10: 3: 12: 4).
Detection: ninhydrin, bioautography by B. subtilis PCI-219.

Detection of Metabolites in Urine (Glycyl-racemomycinic A Acid)

To examine metabolites in urine of ^{14}C-labeled-glycyl-racemomycin-A, urine was collected during 24 hours after the administration. The definite quantity of the collected urine was put to spot tests on the filter paper (Toyo Roshi No. 51). Paper chromatography was performed under the conditions shown in Fig. 3. A peak of strong radioactivity was found at the location of *Rf* value of near the origin, and the location was very low when the antibiotics were taken into account. The location of this peak was the same as the location of *Rf* value of glycyl-racemomycinic A acid, which was produced when lactam ring of the sample of glycyl-racemomycin-A was broken. These findings accounted for the fact that metabolites of streptothricin antibiotics in urine was acid, the lactam ring of which was broken.

Distribution of ^{14}C-Glycyl-Racemomycin-A in Organs of Mice

Distribution of ^{14}C-glycyl-racemomycin-A in mice after intravenous injection of the antibiotic was examined and result is shown in Table II. Table II indicated that it was distributed in the kidney in a high concentration in comparison with the distribution in the other organs.

Table II. Distribution of ^{14}C-Glycyl-Racemomycin-A in Organs of Mice

Organs	Hours (dpm/g)				
	1	3	5	24	48
Brain	211.55	78.95	117.81	289.35	60.55
Heart	272.46	187.84	90.20	175.08	172.40
Liver	791.04	805.72	868.91	718.30	423.10
Lung	892.57	631.71	655.33	280.50	181.11
Kidney	14763.75	16493.50	15204.61	6094.44	2947.20
Spleen	1494.06	1572.09	2007.20	498.70	351.10
Testicle	298.25	137.68	203.14	148.25	74.55
Bladder	1344.10	611.33	601.50	301.80	10.50
Stomach[a)]	430.00	439.09	778.24	470.24	145.22
Small intestine[a)]	331.90	377.20	608.28	329.91	145.22
Large intestine[a)]	345.62	493.12	363.85	328.61	141.72
Urine	568430.11			86898.57	32295.00
Body weight	29.5	29.5	29.0	26.5	28.0

a) A part of extracted organs.

Discussion

In preceding paper,[5)] the authors studied the pharmacokinetics of racemomycin-A and-D belonging to the group of streptothricin, in terms of antibacterial activity. The results showed that high concentration of the antibiotics were distributed in the kidney, and that racemomycin-A might probably be inactivated and excreted into urine. However, it is impossible to say that the distribution of the antibiotics of high concentration in the kidney is always responsible for the renal toxicity. Therefore, pathohistological study were carried out on the liver and spleen which decreased remarkably the weight as reported in the preceding report,[5)] and on the kidney which was whitened macroscopically.

As a result, no pathohistological change was observed except atrophy of the liver and spleen. This also was attested by the fact that plasma GOT and GPT values were in the range of normal value after racemomycin-D was administered to rats. However, severe toxicity wide-sread in the cortex renis was observed in the kidney. This was attested by the macroscopic animals'behavior such as crouching and by the fact that excretion of urine was suspended for four days after the administration of racemomycin-D. These findings led us to a conclu-

sion that streptothricin antibiotics have renal toxicity in a similar manner to the group of streptomycin-neomycin.[9]

As to the distribution of ^{14}C-labeled glycyl-racemomycin-A in the organs of mice, studies confirmed that it was distributed in the kidney in a high concentration in comparison with the distribution in the other organs (Table II).

This finding was substantiated by one of the authors report[5] that the distribution of high concentration of racemomycin-A and-D was observed in the kidney in terms of antibacterial activity.

The concentration in blood showed a very low value one hour after the administration. This finding coincided with the value of the concentration in blood in the time course of one hour, in terms of antibacterial activity, and also it suggests that the transmission to the organ as well as the excretion from the organ are rapid.

When ^{14}C-labeled-glycyl-racemomycin-A was investigated in regard to the metabolism in urine during 24 hours after the administration, its specific radioactivity value in the excreted urine was found as high as 86898.57 dpm/g 24 hours after the administration, but the recovery rate of racemomycin-A in the urine was as low as 8%, in the terms of antibacterial activity.[5] The findings suggest that racemomycin-A might probably be excreted into urine as an inactivated substance. Accordingly, the authors examined the metabolites in urine during 24 hours after the administration of ^{14}C-labeled-glycyl-racemomycin-A.

When paper chromatography of the collected urine was performed, the obtained radiochromatogram showed that the strong radioactivity-peak of the metabolites coincided with the *Rf* value of glycyl-racemomycinic A acid resulting from the breake of lactam ring of which was broken. It is reported that generally streptothricin antibiotics are transformed into acid,[10] lactam ring of which was broken, with one normal solution (1 N) acid and alkali at a room temperature. Further, it is confirmed that acid has no antibacterial activity, and that the toxicity of this acid increase six times that of the original antibiotic. The recovery rate of racemomycin-A in urine during 24 hours was found as low as 8%, but the specific radioactivity of ^{14}C-labeled-glycyl-racemomycin-A was high in the excreted urine. In view of these facts and the examined metabolites in urine, it was proved that administered streptothricin antibiotics were excreted as acid of the inactivated substance.

As to the developmental mechanism of the delayed toxicity, the authors presented the assumption that after the administered antibiotic is, *in vivo*, transformed into acid, lactam ring of which is broken, that acid is responsible for the delayed toxicity.

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