

DISTRIBUTION AND EXCRETION OF CYCLOCYTIDINE IN MONKEYS, DOGS, AND RATS

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The distribution in tissues and excretion of cyclocytidine (2,2'-anhydro-1- β -D-arabinofuranosylcytosine hydrochloride) and its metabolites in urine and feces of macaca monkeys (*Macaca irus*, *Macaca fuscata*, and *Macaca mulata*) and in beagle dogs were examined by the spectrophotometric assay. Distribution of cyclocytidine in plasma and tissues of rats was also examined.

The administered cyclocytidine showed a half-life of 22 min in plasma of dogs and monkeys, whereas the half-life of aracytidine (1- β -D-arabinofuranosylcytosine hydrochloride) was 47 min in plasma of dogs and less than 5 min in plasma of monkeys, because of rapid deamination of the compound to arauridine (1- β -D-arabinofuranosyluracil) in the latter species. Cyclocytidine exhibited maximum concentration in tissues of rats and monkeys at 20 to 40 min after the administration, but its metabolites, aracytidine and arauridine, were not detected in these tissues. Cyclocytidine levels in tissues diminished thereafter but were detected within the next 40 to 80 min. Neither cyclocytidine nor its metabolites could be detected in the brain. When cyclocytidine was administered intravenously in dogs and monkeys, 65~85% of it was excreted in urine, almost all as intact cyclocytidine, and small amounts of aracytidine and arauridine were detected. On the other hand, the administered aracytidine was excreted only as arauridine in urine of monkeys, and aracytidine and arauridine in dogs. Cyclocytidine and its metabolites were not detected in feces of both species.

It might be suggested that the distribution and elimination rate of cyclocytidine after its intravenous administration is not affected by the presence of cytidine deaminase in plasma and tissues.

The remarkable antitumor activity of cyclocytidine (2,2'-anhydro-1- β -D-arabinofuranosylcytosine hydrochloride) against L-1210 leukemia and a variety of mouse tumors was found by Hoshi *et al.*^{10, 11)} and by other workers.^{16, 23)} Furthermore, cyclocytidine was found to be effective in leukemia patients at phase I study.²⁰⁾ Pharmacological and toxicological properties of the compound in mice, rats, monkeys, and dogs were examined by Hirayama *et al.*^{7~9)} The side effect of cyclocytidine is rare and its cumulative toxicity is extremely low in laboratory animal species, when compared with other antitumor agents such as aracytidine (1- β -D-arabinofuranosylcytosine hydrochloride).^{7~9)} It was reported that aracytidine was inactivated by cytidine deaminase through its deamination to arauridine (1- β -D-arabinofuranosyluracil) when used clinically.^{4, 5, 6, 18, 22)} Cytidine deaminase activity in macaca monkey organs as well as in human organs is in a relatively high level, whereas that in dog and rat organs is considerably low or none.^{1, 2, 3, 18)} Cyclocytidine was found to be resistant against cytidine deaminase.^{9, 12, 19)} It is, therefore, important in the clinical application of cyclocytidine to investigate the distribution and excretion in experimental animals having a high deaminase activity.

The present study deals with the distribution and excretion of cyclocytidine and its metabolites after intravenous administration of cyclocytidine in macaca monkeys, beagle dogs, and also distribution in rat tissues.

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MATERIALS AND METHODS

Chemicals Cyclocytidine was synthesized by the method of Kanai *et al.*¹³⁾ and of Kikugawa *et al.*¹⁵⁾ The compound occurs as white needles of mp 255–257°, easily soluble in water but slightly soluble in ethanol, methanol, and acetone, and almost insoluble in ether, chloroform, and benzene. Chemical structure of cyclocytidine and aracytidine are shown in Fig. 1.

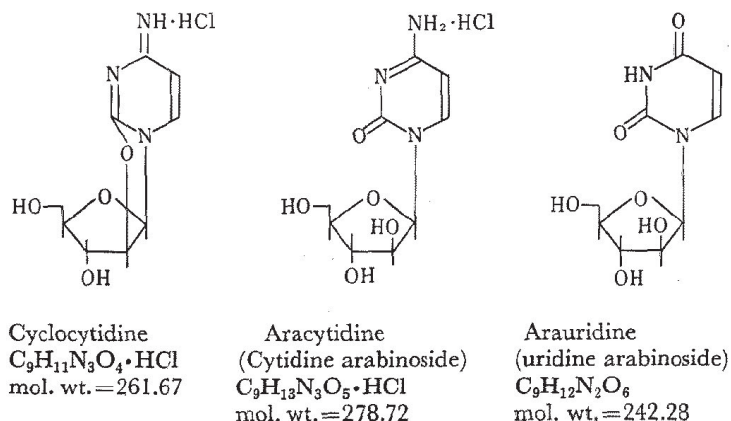


Fig. 1. Chemical structures

Animals Macaca monkeys (*Macaca irus*, *Macaca mulata*, and *Macaca fuscata*), weighing 2–7 kg (Kyudoh Co., Kumamoto), beagle dogs weighing 8–14 kg (Laboratory for Experimental Beagle Dog, Nippon Dog Center Co., Kagoshima), and rats of Wistar strain weighing 180–200 g (Kyudoh Co., Kumamoto) were divided into 3 to 6 groups. Both males and females of monkeys and dogs were kept in individual cages fed on a commercial diet; Type AF (Oriental Yeast Co., Tokyo) for monkeys and Vita-One (CLEA Japan Inc., Osaka) for dogs twice a day, with vegetables to monkeys and meat to dogs. Water was given freely from bottles. Rats were allowed free access to a commercial diet, Type MF (Oriental Yeast Co., Tokyo) and fresh water. All the animals were kept in air-conditioned rooms at $24 \pm 1^\circ$ and $55 \pm 5\%$ relative humidity, and kept for 4–6 weeks before each experiment.

Cyclocytidine and aracytidine were dissolved in physiological saline and intravenously administered to monkeys via the median antebrachial vein and to dogs via the saphenous vein. Control animals received physiological saline alone.

Collection and Preparation of Samples Blood samples were collected from 12 monkeys via the median antebrachia vein, from 13 dogs via the saphenous vein, and from rats via the femoral vein 20, 40, 80, or 160 min after the administration of cyclocytidine. Visceral specimens were also obtained from the same animals at the above described time. Urine samples were collected 10, 20, 80, 160, or 320 min and 12, 24, 48, or 72 hr after the administration, and stools spontaneously evacuated for 4 days were also collected. These blood, organ, urine, and fecal samples were treated as described below and subjected to spectrophotometric assay.¹⁴⁾

Plasma Plasma was separated from the heparinized blood by centrifugation (3,000g, 4° , 15 min) and deproteinized with trichloroacetic acid at the final concentration of 4%.

Organs Monkeys and rats were sacrificed by bleeding under ether anesthesia and vascular perfusion with Ringer solution was carried out with a peristal pump (Taiyo Rika Kikai Co., Tokyo) to remove residual blood. The abdomen was immediately opened, and brain, salivary gland, muscle, liver, kidneys, spleen, and other visceral organs were removed and weighed individually. A portion of these tissues was minced and homogenized with 3–4 volumes of 4% cold trichloroacetic acid by the Potter-Elvehjem type Teflon homogenizer or a cutting-blade homogenizer in ice-cold bath and the supernatant was separated by centrifugation.

Urine and Feces From monkeys and dogs housed individually in metabolism cages, urine samples were collected in plastic containers containing acetic acid and spontaneously evacuated stools obtained as described above. Both urine and feces were also treated with 4% trichloroacetic acid solution.

Separation of Cyclocytidine, Aracytidine, and Arauridine The samples were fractionated according to the procedure of Kanai *et al.*¹⁴⁾ into three fractions; A (auridine), B (aracytidine), and C (cyclocytidine), as shown in Fig. 2. Each 2 ml of the sample was applied on a column (1.4 × 3.4 cm) of Dowex 50W × 4 (NH_4 form) with a Chromacord-UV Analyzer (Model UV-254, Shibata Chemical Apparatus Mfg. Co., Osaka). The first fraction-A was eluted with water, concentrated *in vacuo* at 40° , and chromatographed

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on Toyo Roshi No. 51A filter paper for 10~12 hr, using a solvent system of ethyl acetate: isopropanol: water=65:17.5:17.5 (v/v). The UV-absorbing spot was identified with authentic arauridine. After elution with water, amount of arauridine was determined by spectrophotometry at 263 nm. The second fraction-B was eluted from the column with 0.4M ammonium acetate. After concentration *in vacuo* at 40°, aracytidine was separated by an ascending paper chromatography with a solvent system of 5M ammonium acetate: water-saturated sodium borate: ethanol:0.5M EDTA disodium salt = 20:80:220:0.5 (v/v). The amount of aracytidine was determined by UV absorption in 0.1N HCl at 280 nm. Finally, fraction-C containing cyclocytidine was eluted with 0.4M ammonium acetate and its quantity was assayed by UV absorption in 0.4M ammonium acetate at 263 nm.

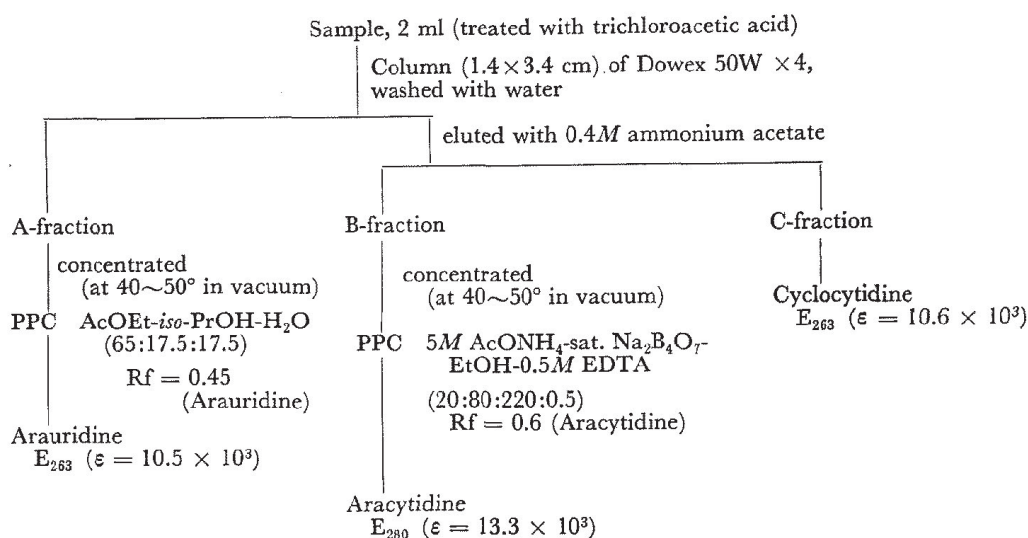


Fig. 2. Fractionation procedure for cyclocytidine, aracytidine, and arauridine

RESULTS

Half-lives of Administered Cyclocytidine and Aracytidine in Plasma of Monkeys, Dogs, and Rats
 Monkeys: As shown in Fig. 3, expected metabolites were not detected in plasma of monkeys after the intravenous administration of 100 mg/kg of cyclocytidine. The plasma cyclocytidine levels in monkeys is shown in Fig.3. Cyclocytidine was present in approximately 170 $\mu\text{g/ml}$ in plasma immediately after the injection, and it fell rapidly and disappeared within 80 min. The half-life of cyclocytidine was, therefore, determined as 22 min. This value was not different among the three simian species, cynomolgus, rhesus, and fuscata.

The plasma of monkeys contained 50 $\mu\text{g/ml}$ of aracytidine immediately after receiving 100 mg/kg of aracytidine, but a drastic increase of its metabolite, 100~400 $\mu\text{g/ml}$ of arauridine, in plasma occurred already 5 min later. Aracytidine disappeared completely from the plasma within 10 min and arauridine alone was detected. Arauridine diminished gradually as shown in Fig. 4. The half-life of aracytidine was therefore considered to be less than 5 min in the three simian species.

Dogs: As shown in Fig.5, only cyclocytidine was detected in plasma after the administration of 100 mg/kg of cyclocytidine, and its concentration in plasma was about 200 $\mu\text{g/ml}$ after 10 min and disappeared within 80 min, the half-life of cyclocytidine being 22 min. At 10 min after injection of the same dose of aracytidine, plasma level of aracytidine was 100 $\mu\text{g/ml}$ and it decreased gradually. Arauridine was not detected in plasma (Fig. 5). The half-life of aracytidine was 47 min in dogs. These findings indicate that the disappearance of aracytidine from plasma was gradual in the animals having low or no cytidine deaminase activity.

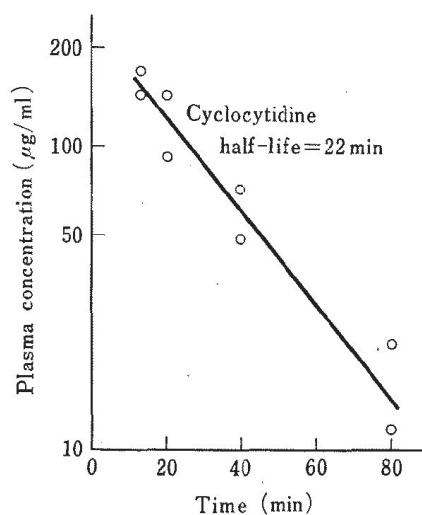


Fig. 3. Plasma level of cyclocytidine in macaca monkeys after intravenous administration of 100 mg/kg of cyclocytidine

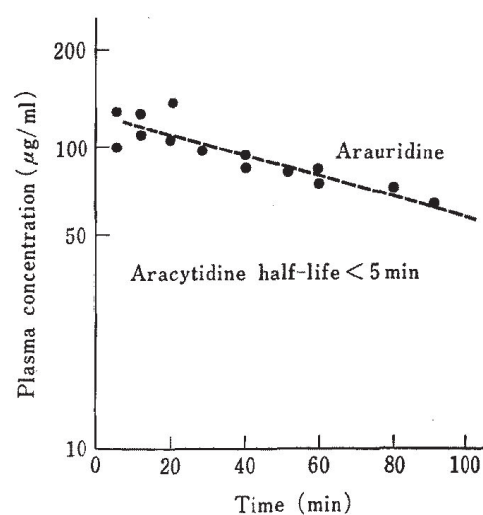


Fig. 4. Plasma level of arauridine in macaca monkeys after intravenous administration of 100 mg/kg of aracytidine

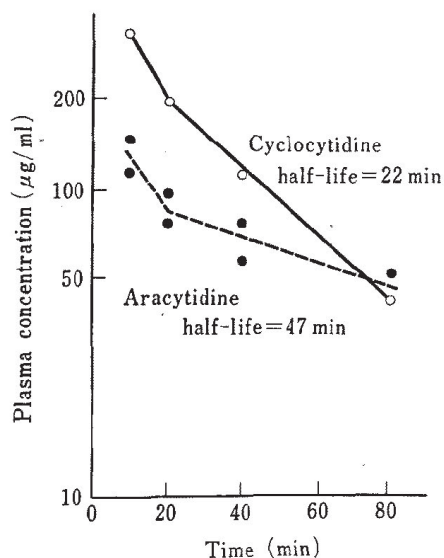


Fig. 5. Plasma level of cyclocytidine and aracytidine in dogs after intravenous administration of 100 mg/kg of cyclocytidine or aracytidine

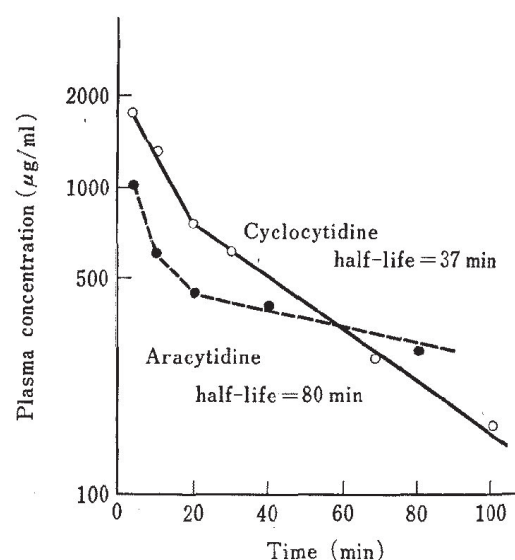


Fig. 6. Plasma level of cyclocytidine and aracytidine in rats after intravenous administration of 100 mg/kg of cyclocytidine

Rats: As shown in Fig. 6, neither aracytidine nor arauridine was detected in plasma after the administration of 600 mg/kg of cyclocytidine, and intact cyclocytidine alone was detected in plasma. The level of cyclocytidine was 1,800 $\mu\text{g/ml}$ in plasma 5 min after the injection, diminished to 800 $\mu\text{g/ml}$ within 20 min, and continued to decrease during the next 100 min. Half-life of cyclocytidine in plasma of rats was about 37 min and that of aracytidine, 80 min.

Distribution in Tissues Monkeys: Figs. 7 and 8 illustrate the serial plasma levels and distribution of cyclocytidine in tissues of monkeys after the intravenous injection of 600 mg/kg of cyclocytidine. Fig. 7 shows the pattern of plasma level, and about 1,000 $\mu\text{g/ml}$ of cyclocytidine

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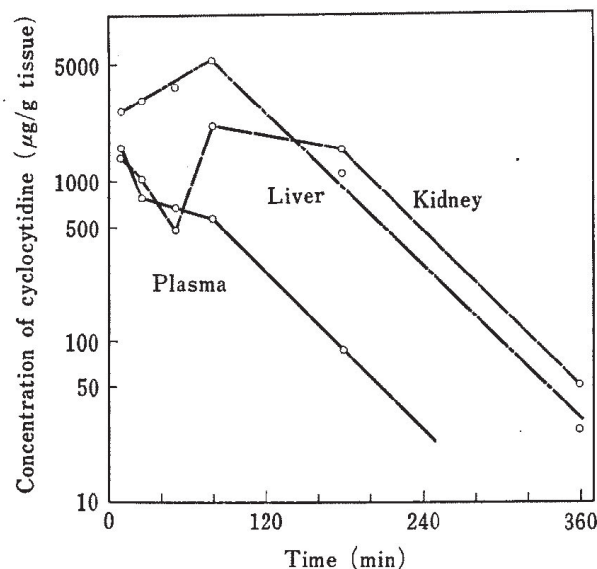


Fig. 7. Distribution of cyclocytidine in plasma, liver, and kidney of monkeys after intravenous administration of 600 mg/kg of cyclocytidine

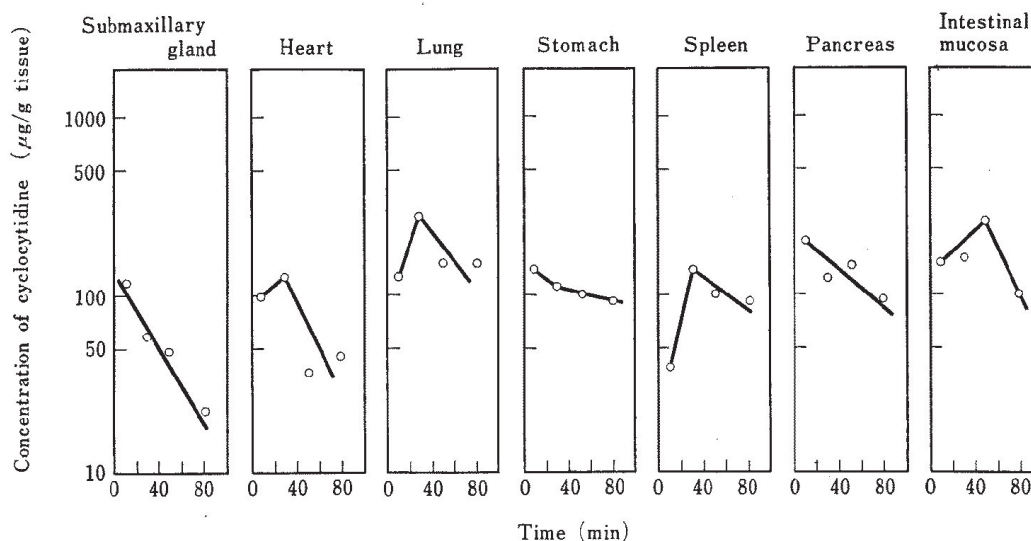


Fig. 8. Distribution of cyclocytidine in monkey tissues after intravenous administration of 600 mg/kg of cyclocytidine

was demonstrated 5 min after the injection of cyclocytidine, but arauridine and aracytidine were not detected. Cyclocytidine decreased gradually and was detected even 240 min later. Maximum concentrations of cyclocytidine in liver and kidney were 5,000 and 2,500 $\mu\text{g/g}$, respectively, after 80 min, and concentration decrease was like that in plasma. Fig. 8 shows the distribution patterns in other tissues and significant amount of cyclocytidine was found during the initial 20 to 40 min in pulmonary tissue and intestinal mucosa (300~400 $\mu\text{g/g}$). Spleen and cardiac tissues exhibited a notable uptake (100~200 $\mu\text{g/g}$) in the first 20 min and a rapid uptake (100 $\mu\text{g/g}$) also occurred in pancreas and salivary gland. The compound disappeared from the tissue progressively in the subsequent period of 80 min. Brain tissues failed to show any significant detectable level of cyclocytidine.

Rat: The metabolites, aracytidine and arauridine, were not found in any tissues and plasma of rats after 600 mg/kg of cyclocytidine was given intravenously. As seen in Fig. 9, the maximum concentration of cyclocytidine in plasma was 1,000 $\mu\text{g/g}$ during the initial 80 min. Renal tissue maintained a high concentration of over 1,000 $\mu\text{g/g}$ of cyclocytidine in the first 40 min, which declined slowly in parallel with the plasma concentration. The compound in lung and salivary gland increased in the first 20 min and then it disappeared completely during the following 40 to 100 min. Maximum concentration of 300~500 $\mu\text{g/g}$ of cyclocytidine was seen in the stomach, intestine, and pancreas immediately after its administration and subsequently disappeared within 80 to 100 min. Cyclocytidine was not detected in brain throughout this experimental period.

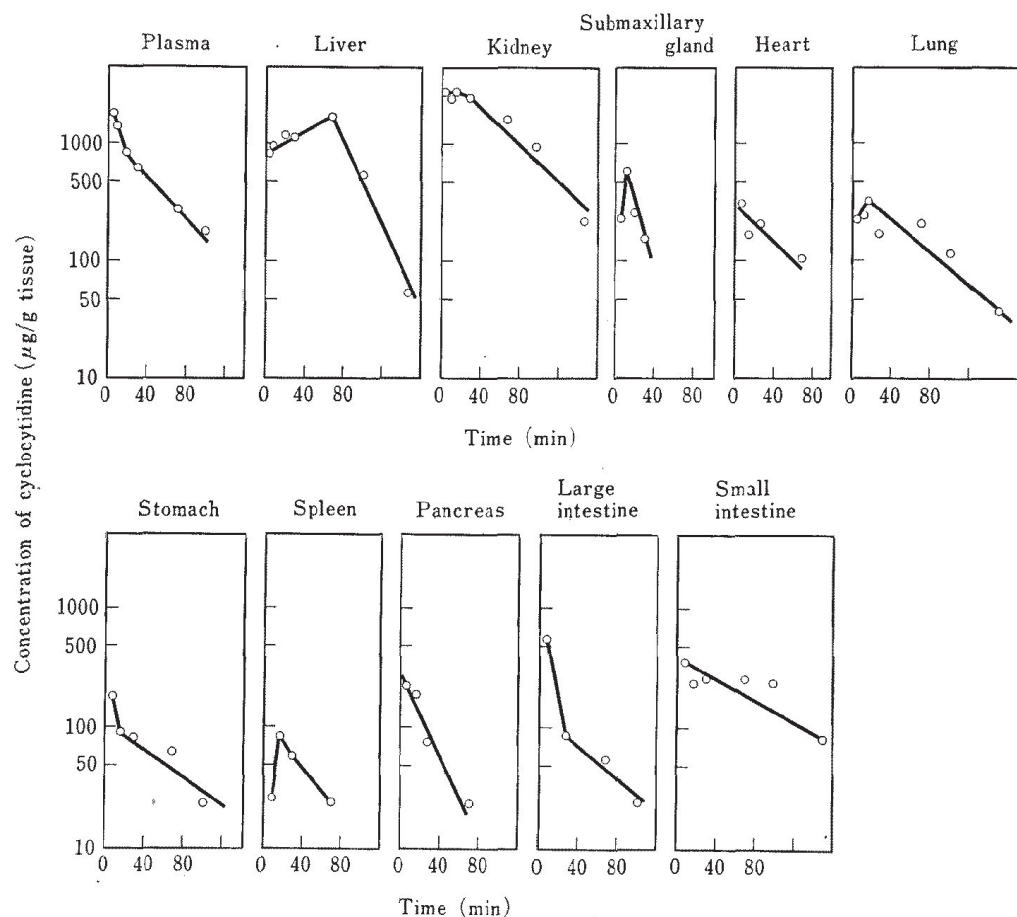


Fig. 9. Distribution of cyclocytidine in rat tissues after intravenous administration of 600 mg/kg of cyclocytidine

Urinary Excretion of Cyclocytidine, Aracytidine, and Arauridine in Dogs and Monkeys Monkeys: Table I shows the urinary excretion pattern after the administration of 100 mg/kg of cyclocytidine in monkeys. The amount of excreted material was 40.9~64.5% as cyclocytidine, 0~0.3% as aracytidine, and 1.9~2.0% as arauridine during 4 hr after the injection. Moreover, 7.1~9.3% was excreted as cyclocytidine, 0~1.3% as aracytidine, and 1.8~1.9% as arauridine within the following 24 hr, and they were not detected after that time. On the other hand, the injected aracytidine was excreted very rapidly as arauridine which was the sole metabolite detected at the rate of 34.7% and 13.2% in 4-hr and 8-hr urine, respectively (Table II).

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Table I. Excretion Ratio of Cyclocytidine and Its Metabolites in Urine of Macaca Monkeys after Intravenous Administration of 100 mg/kg of Cyclocytidine

Time (hr)	Case 1 (female, 2.8 kg)			Case 2 (female, 3.4 kg)		
	Cyclocytidine	Aracytidine	Arauridine	Cyclocytidine	Aracytidine	Arauridine
	(%)	(%)	(%)	(%)	(%)	(%)
4	64.5	0	1.9	40.9	0.3	2.0
8	—	—	—	3.4	0.3	1.4
24	9.3	0	1.9	3.7	1.0	0.4
48	0	0	0	0	0	0
Total	73.8	0	3.8	48.0	1.6	3.8
	77.6			53.4		

Dogs: In urine of dogs collected for 56 hr after the intravenous administration of 100 mg/kg of cyclocytidine, about 80% was excreted, 65% as clcyocytidine, 15% as aracytidine, and 1.5% as arauridine (Table III). In urine of dogs administered with aracytidine, 24.2% was excreted as aracytidine and 5% as arauridine (Table IV).

These results indicated that in macaca monkeys and dogs, cyclocytidine was excreted in urine mainly within 24 hr after the administration at 67 and 74% as cyclocytidine, 0 and 18% as aracytidine, and 2 and 4% as arauridine, respectively. There was a difference between these two species after aracytidine administration, and administered aracytidine was excreted 48% as arauridine in monkeys, and 6% as arauridine and 24% as aracytidine in dogs.

Table II. Excretion Ratio of Aracytidine and Arauridine in Urine of Macaca Monkey after Intravenous Administration of 100 mg/kg of Aracytidine

Time (hr)	Case 1 (female, 3.2 kg)	
	Aracytidine	Arauridine
	(%)	(%)
4	0	34.7
8	0	13.2
24	0	0
48	0	0
Total	0	47.9
	47.9	

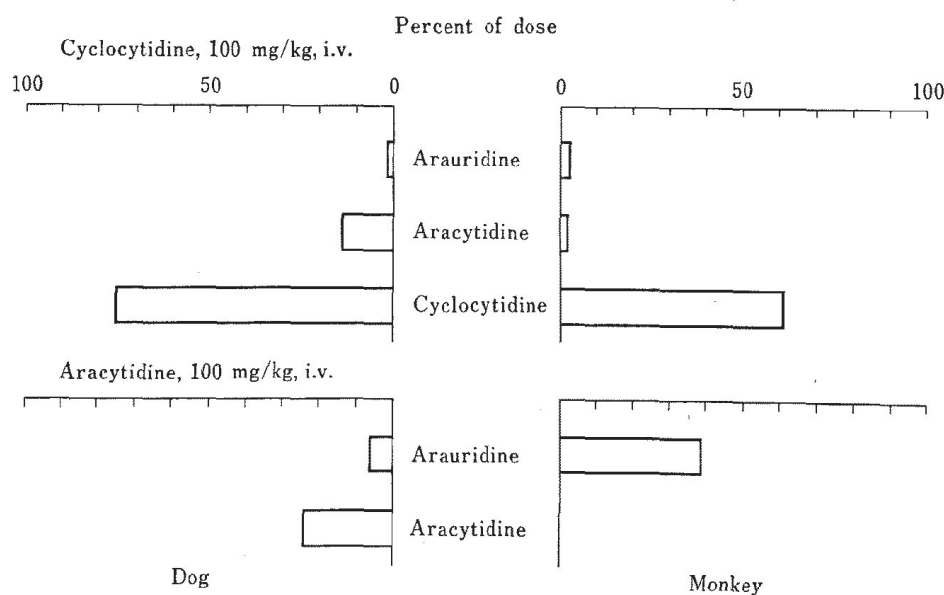


Fig. 10. Cumulative urinary excretion in dogs and monkeys as percentage of dose at 56 hr after single intravenous administration of 100 mg/kg of cyclocytidine

Table III. Excretion Ratio of Cyclocytidine and Its Metabolites in Urine of Beagle Dogs after Intravenous Administration of 100 mg/kg of Cyclocytidine

Time (hr)	Case 1 (female, 10 kg)			Case 2 (female, 10.5 kg)		
	Cyclocytidine	Aracytidine	Arauridine	Cyclocytidine	Aracytidine	Arauridine
	(%)	(%)	(%)	(%)	(%)	(%)
4	—	—	—	47.1	3.3	0.5
10	—	—	—	17.2	4.0	1.0
24	59.7	13.3	1.5	2.9	2.7	0
32				0	0	0
48	2.3	3.7	0			
54	1.8	1.8	0			
Total	63.8	18.8	1.5	67.2	10.0	1.5
		84.1			78.7	

Table IV. Excretion Ratio of Aracytidine and Arauridine in Urine of Beagle Dogs after Intravenous Administration of 100 mg/kg of Aracytidine

Time (hr)	Case 1 (female, 11.2 kg)		Case 2 (female, 8.7 kg)	
	Aracytidine	Arauridine	Aracytidine	Arauridine
	(%)	(%)	(%)	(%)
4	13.2	2.8	22.3	4.5
6	3.3	0.7	1.9	1.0
24	1.7	0.7	0	0.5
32	2.1	0.2	0	0.3
48	3.2	0.3		
56	0.7	0.1		
Total	24.2	4.8	24.2	6.3
	29.0		30.5	

Excretion of Cyclocytidine and Aracytidine in Feces of Monkeys Cyclocytidine, aracytidine, and arauridine were not detected in the feces evacuated within 72 hr after the intravenous administration of 100 mg/kg of cyclocytidine or aracytidine.

DISCUSSION

Present experiments were carried out in monkeys, dogs, and rats to investigate the species difference in distribution and metabolism of cyclocytidine in plasma and tissues, and of its excretion in urine and stool.

After the administration of cyclocytidine, neither aracytidine nor arauridine was detected in the circulating plasma and only intact cyclocytidine was detected in monkeys and dogs. Cyclocytidine levels in plasma did not differ significantly between these two animal species and the biological half-life was 22 min, while it was 37 min in rats, which suggested that cyclocytidine is resistant either to cytidine deaminase or other metabolizing enzymes *in situ*. On the other hand, the biological half-life of aracytidine was found to be 47 and 80 min in dogs and rats, respectively, whereas it was less than 5 min in monkeys, because of its metabolism to arauridine by cytidine deaminase activity.^{1, 2, 3, 24)} These results indicated that cyclocytidine was scarcely affected by cytidine deaminase in plasma and confirm the results of *in vitro* experiments previously reported by Hoshi *et al.*¹²⁾ As for metabolites of cyclocytidine in tissues, neither aracytidine nor arauridine was detected in liver, kidney, lung, salivary gland, or gastrointestinal mucosa. Cyclocytidine in these tissues diminished in parallel with its reduction in plasma. Of considerable interest was the fact that the compound distributed in high concentra-

tion in the salivary gland and gastrointestinal. This agreed with our previous findings that cyclocytidine acted pharmacologically to cause hypersalivation and mild inhibitory action on the spontaneous gastrointestinal motility.^{7,8)} These data might suggest the clinical applicability of cyclocytidine as a chemotherapeutic agent against tumors of salivary gland and gastrointestinal. Meanwhile, no evidence was obtained for the distribution of cyclocytidine in brain tissues, with the lack of significant pharmacological effect of the compound on the central nervous system.^{8,21)}

As summarized in Fig. 10, about 65% of intact cyclocytidine was excreted in urine within 56 hr in monkeys and about 65% within 56 hr in dogs, though the deaminase activity in plasma and tissues is different in the two animal species. A slight excretion of arauridine, which is a deaminated metabolite through aracytidine, was greater in monkeys than in dogs. On the other hand, in the administration of aracytidine, it was excreted 24.2% as aracytidine and 4.8~6.3% as arauridine within 56 hr in dogs, but it was not detected as aracytidine and 47.9% of it was excreted as arauridine within 8 hr in monkeys. These data indicated that most of administered cyclocytidine was not metabolized rapidly to arauridine through aracytidine. It might account for the lower cumulative toxicity of cyclocytidine than that of aracytidine in dogs and monkeys.⁹⁾ The distribution and elimination of cyclocytidine in mice and macaca monkeys have been examined using ¹⁴C- or ³H-labeled compounds, and its results will be published elsewhere.

(Received November 7, 1973)

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