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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 Cycloctidine 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 cycloctidine and aracytidine are shown in Fig. 1.

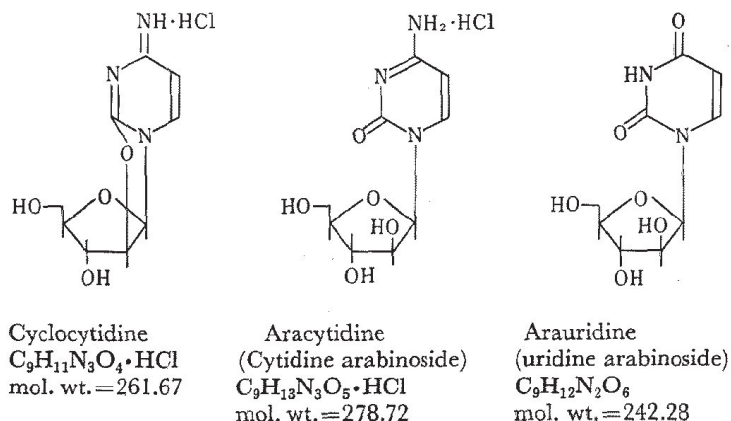


Fig. 1. Chemical structures

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 $24^\circ \pm 1^\circ$ and $55 \pm 5\%$ relative humidity, and kept for 4~6 weeks before each experiment.

Cycloctidine 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 cycloctidine. 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 Cycloctidine, Aracytidine, and Arauridine The samples were fractionated according to the procedure of Kanai *et al.*¹⁴⁾ into three fractions; A (arauridine), B (aracytidine), and C (cycloctidine), 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.

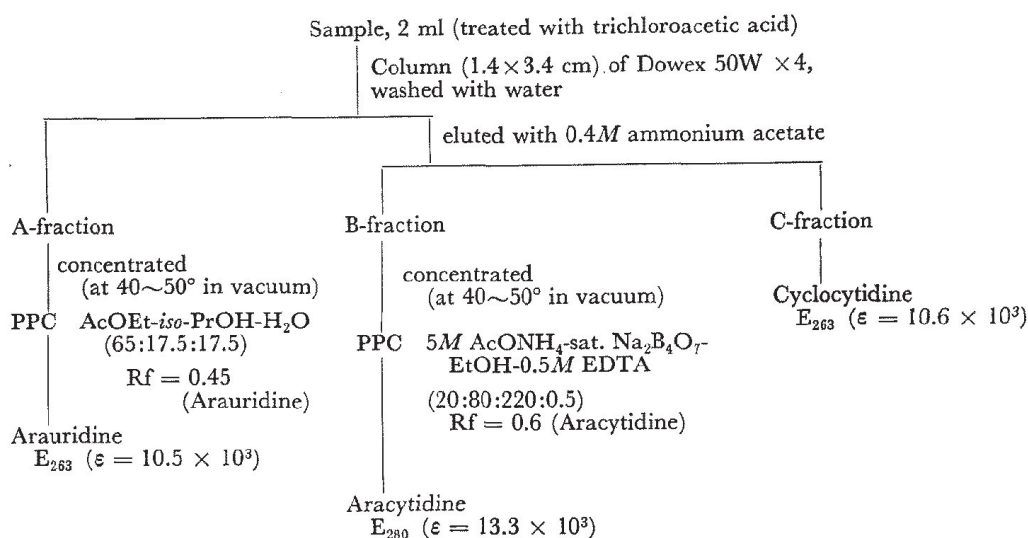


Fig. 2. Fractionation procedure for cyclocytidine, aracytidine, and arauridine

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.

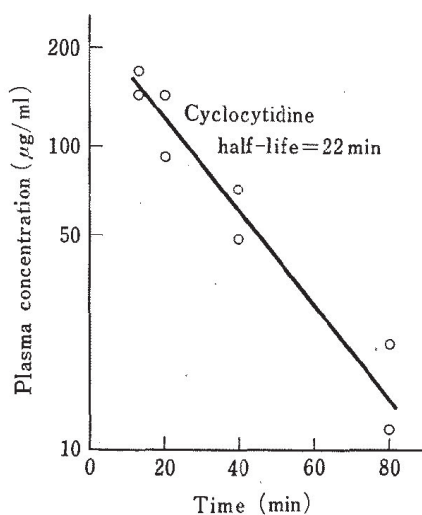


Fig. 3. Plasma level of cycloctidine in macaca monkeys after intravenous administration of 100 mg/kg of cycloctidine

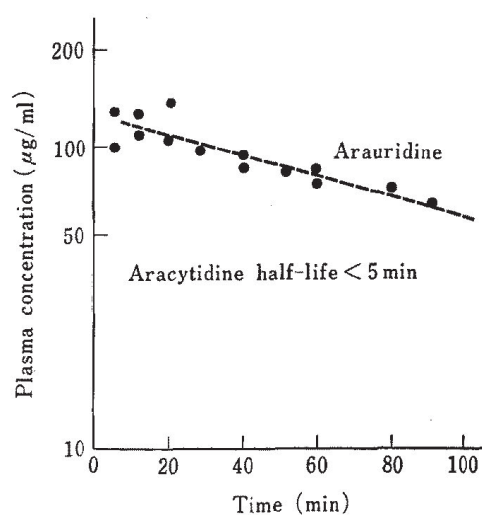


Fig. 4. Plasma level of arauridine in macaca monkeys after intravenous administration of 100 mg/kg of aracytidine

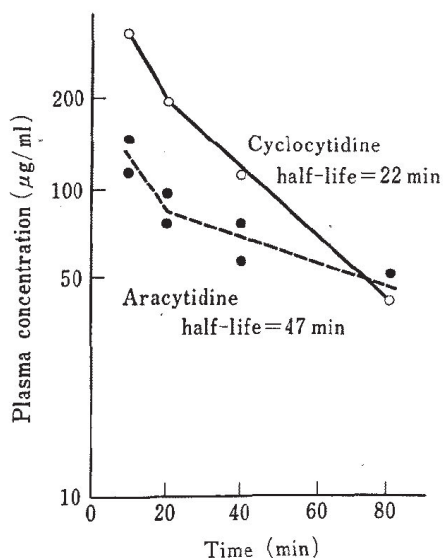


Fig. 5. Plasma level of cycloctidine and aracytidine in dogs after intravenous administration of 100 mg/kg of cycloctidine or aracytidine

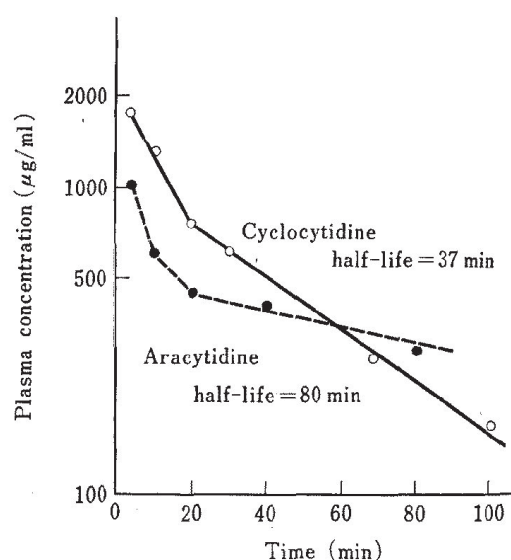


Fig. 6. Plasma level of cycloctidine and aracytidine in rats after intravenous administration of 100 mg/kg of cycloctidine

Rats: As shown in Fig. 6, neither aracytidine nor arauridine was detected in plasma after the administration of 600 mg/kg of cycloctidine, and intact cycloctidine alone was detected in plasma. The level of cycloctidine was 1,800 µg/ml in plasma 5 min after the injection, diminished to 800 µg/ml within 20 min, and continued to decrease during the next 100 min. Half-life of cycloctidine 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 cycloctidine in tissues of monkeys after the intravenous injection of 600 mg/kg of cycloctidine. Fig. 7 shows the pattern of plasma level, and about 1,000 µg/ml of cycloctidine

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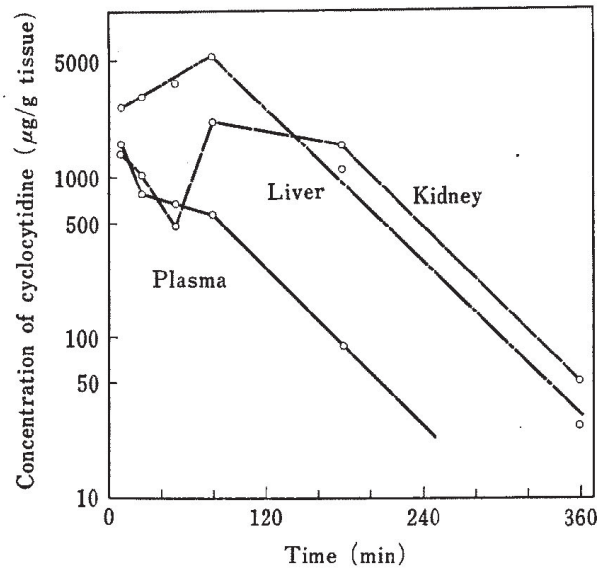


Fig. 7. Distribution of cyclocytidine in plasma, liver, and kidney of monkeys after intravenous administration of 600 mg/kg of cyclocytidine

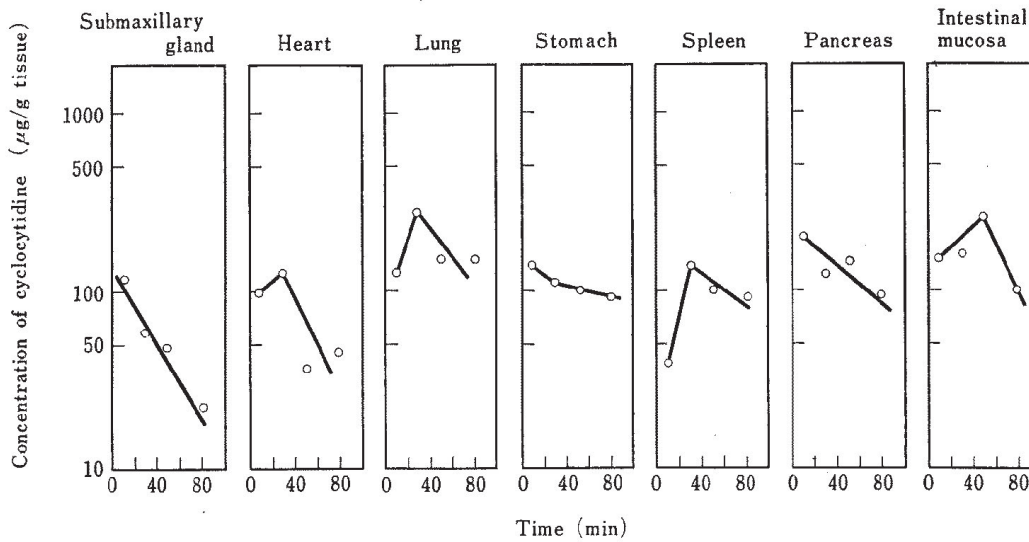


Fig. 8. Distribution of cyclocytidine in monkey tissues after intravenous administration of 600 mg/kg of cyclocytidine

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.

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