[Chem. Pharm. Bull. 23(8)1814—1818(1975)]

UDC 547.963.32.09'615.31.033.076.9

# Metabolism of Cyclocytidine and Aracytidine in Mice[1]

Akio Hoshi, Mitsuzi Yoshida, Kazuo Kuretani,[2a] Tadashi Kanai and Motonobu Ichino[2b]

*Pharmacology Division, National Cancer Center Research Institute[2a] and Research Laboratories, Kohjin Co., Ltd.[2b]*

(Received December 26, 1974)

Rate of excretion and metabolites in urine and feces of mice after intravenous or oral administration of $^{14}$C-cyclocytidine and $^{14}$C-aracytidine were examined. Furthermore, absorption from gastrointestinal tract was examined. After intravenous injection, about 80% of cyclocytidine was excreted in urine within 24 hours and not in feces. Main radioactive compound in urine was intact cyclocytidine and small amount of aracytidine and arauridine were detected, while two-thirds of $^{14}$C-aracytidine administered were excreted after metabolized to arauridine. A half of cyclocytidine was excreted in feces as intact when administered orally. Very small amount of cyclocytidine was excreted in expired air as $CO_2$. However, one-fourth of radioactivity was excreted in expired air after oral administration of $^{14}$C-aracytidine. Cyclocytidine was mainly absorbed from stomach and slightly from large intestine.

Cyclocytidine (2,2'-anhydro-1-β-D-arabinofuranosyl cytosine hydrochloride) was markedly active against L 1210 leukemia[3] and a variety of mouse tumors.[4] This compound was also found to be effective clinically in leukemic patients.[5] Furthermore, side effects of cyclocytidine were weak and its cumulative toxicity was very low.[3,6] Therefore, resulted therapeutic ratio in L1210 leukemia cells of the compound was greater than that of aracytidine (1-β-D-arabinofuranosyl cytosine).[3] Cyclocytidine was also active against L1210 cells when administered orally to the mice bearing these leukemia cells, but potency of the compound was decreased to two-thirds of that by intraperitoneal treatment.[7] Metabolites of cyclocytidine in monkeys and dogs were examined by chemical analysis and the results were reported separately.[8]

The present study deals with the rate of excretion and metabolites in urine of mice after intravenous injection or oral administration of $^{14}$C-cyclocytidine.

## Materials and Methods

**Labeled Compounds**——2-$^{14}$C-Cyclocytidine and 2-$^{14}$C-aracytidine were 90% radiochemically pure as assayed by thin-layer chromatography using a solvent system of *n*-propanol: tetrahydrofuryl alcohol:

1) This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education and a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare. Part of this work was presented at the 94th Annual Meeting of Pharmaceutical Society of Japan, Sendai, April 1974.

2) Location: *a) Tsukiji 5-1-1, Chuo-ku, Tokyo, 104, Japan; b) Komiya-cho, Hachioji, Tokyo, 192, Japan.*

3) A. Hoshi, F. Kanzawa, K. Kuretani, M. Saneyoshi, and Y. Arai, *Gann*, **62**, 145 (1971); J.M. Venditti, M.C. Baratta, N.H. Greenberg, B.J. Abbott and I. Kline, *Cancer Chemother. Rep.*, **56**, 483 (1972).

4) A. Hoshi, F. Kanzawa, and K. Kuretani, *Gann*, **63**, 353 (1972); W. Nakahara and R. Tokuzen, *ibid.*, **63**, 379 (1972); R. Tokuzen, K. Kuretani, and W. Nakahara, *ibid.*, **65**, 89 (1974).

5) Y. Sakai, C. Konda, M. Shimoyama, T. Kitahara, T. Sakano, and K. Kimura, *Jap. J. Clin. Oncol.*, **2**, 57 (1972).

6) H. Hirayama, K. Sugihara, K. Wakigawa, J. Hikita, M. Iwamura, and H. Ohkuma, *Pharmacometrics*, **6**, 1255 (1972); H. Hirayama, K. Sugihara, K. Wakigawa, J. Hikita, T. Sugihara, T. Kubota, H. Ohkuma, and K. Oguro, *ibid.*, **6**, 1259 (1972); H. Hirayama, K. Sugihara, T. Sugihara, K. Wakigawa, M. Iwamura, H. Ohkuma, and J. Hikita, *ibid.*, **8**, 353 (1974).

7) A. Hoshi, F. Kanzawa, and K. Kuretani, *Gann*, **63**, 279 (1972).

8) H. Hirayama, T. Sugihara, F. Hamada, T. Kanai, J. Hikita, Y. Arai, K. Kuretani, and A. Hoshi, *Gann*, **65**, 153 (1974).

acetic acid (40: 20: 1). The specific radioactivity of the compounds were 1.5 μCi/mg as cyclocytidine. It was prepared according to the method of Kanai, *et al.*[9]

**Animals**——All experiments were performed using female ddN mice weighing 20±1 g. The commercial diet (CA-1, Nihon CLEA Co., Tokyo) and water offered *ad libitum*.

**Administration of the Compounds**——Radioactive cyclocytidine at 34 mg/kg (1 μCi/mouse) or aracytidine at 36 mg/kg (1 μCi/mouse) in saline was administered intravenously or orally.

**Detection of Cyclocytidine and Its Metabolites**——Thin-layer chromatography was used for detecting of cyclocytidine and its metabolites in urine, feces and gastrointestinal contents. Silica gel $F_{254}$ plates (Merck) were used for thin-layer chromatography. Gastrointestinal contents and feces were homogenized with 20 volumes of 70% ethyl alcohol and centrifuged. Five μl of this supernatant was spotted on the plate and developed with the solvent system consisted of *n*-propanol: tetrahydrofuryl alcohol: acetic acid (40: 20: 1). The percentage of each metabolite was calculated from the peak area of the radioactivity determined by an Aloka TLC Chromatogram Scanner (Model TLC-2B). The spots on the chromatogram were also confirmed by autoradiography using Sakura X-ray film (Type N).

**Collection of Urine and Feces**——The urine and feces of five mice in a group were collected separately 4, 24 and 48 hr after the administration in a metabolic cage. Urine was collected in 0.1 or 1N HCl. The feces were homogenized with 20 volumes of 70% ethyl alcohol as described above. Mean of five specimens was calculated.

**Collection of Carbon Dioxide in Expired Air**——The expired $CO_2$ was trapped in 20 ml each of 20% KOH in a series of four bottles by aspiration of air. A mouse was housed in a metabolic cage which was settled in a vinyl isolater after administration of the radioactive compound at 1 μCi/mouse. Mean of five specimens was calculated.

**Collection of Gastrointestinal Contents**——Gastrointestinal tract was tied and excised 1, 2 and 4 hr after administration. Contents of tract were homogenized with 5% trichloroacetic acid. Five mice in each group were used. Mean of five specimens was calculated.

**Detection of Radioactivity**——Homogenate of gastrointestinal contents in 5% trichloroacetic acid and homogenate of feces in 70% ethyl alcohol were solibilized for overnight with Soluene-100 (Packard) at room temperature. These solubilized samples and urine were added toluenecellosolve scintillator consisted of 5 g PPO, 0.5 g dimethyl POPOP, 600 ml toluene and 400 ml ethylcellosolve. The expired $CO_2$ which was trapped in 20% KOH was diluted with 4 volumes of water and added toluene-Triton scintillator consisted of 5.5 g PPO, 0.1 g dimethyl POPOP, 666 ml toluene, 333 ml Triton-X 100 and 70 ml of water. Radioactivity was determined with a Packard Tri-Carb 3320 liquid scintillation spectrometer. Quenching was corrected by an external standard method.

## Results and Discussion

### Rate of Excretion after Intravenous Administration

As shown in Fig. 1, about 60 and 70% of total radioactivity were excreted in urine within 4 hours after intravenous administration of $^{14}$C-cyclocytidine and $^{14}$C-aracytidine, respectively. Excretion of radioactivity reached to 80% 24 hours after administration in both compounds. Excretion rate in cyclocytidine was slower than that in aracytidine during initial 4 hours. These observations were agreed with the results obtained from experiments concerning to distribution of cyclocytidine in tissues with time.[10] No radioactivity was detected in feces during 24 hours.

### Metabolites in Urine after Intravenous Administration

Metabolites in urine after intravenous injection of $^{14}$C-cyclocytidine and $^{14}$C-aracytidine were examined by thin-layer chromatography. As shown in Fig. 2 and 3, four radioactive spots (*Rf* 0, 0.12, 0.67 and 0.83) were detected on the chromatograms in urine after administration of $^{14}$C-cyclocytidine. Three spots were identified as cyclocytidine (0.12), aracytidine (0.67) and arauridine (1-β-D-arabinofuranosyl uracil: 0.83) but one spot (0) was unknown.

Main part (85%) of radioactive compounds in urine was intact cyclocytidine. Small amount of aracytidine which was the transformed product of cyclocytidine and of arauridine

9) T. Kanai, T. Kojima, O. Maruyama, and M. Ichino, *Chem. Pharm. Bull.* (Tokyo), **17**, 848 (1970).
10) A. Hoshi, M. Iigo, K. Kuretani, T. Kanai, and M. Ichino, *Chem. Pharm. Bull.* (Tokyo), **23**, 725 (1975).

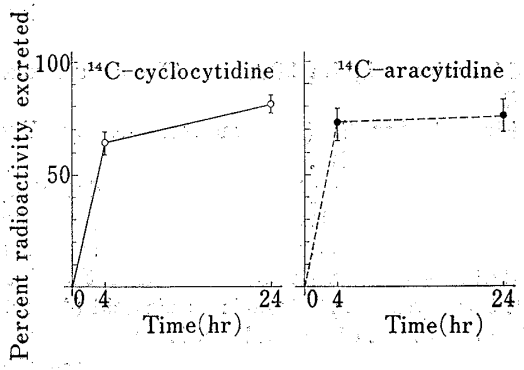


Fig. 1. Excretion of Radioactivity in Urine of Mice after Intravenous Injection of $^{14}$C-Cyclocytidine and $^{14}$C-Aracytidine

The bars indicate standard errors of the mean for five specimens

which was a further deaminated product of aracytidine were detected in 0—4 hr urine. Ratio of radioactive metabolites in 4—24 hr urine was similar to that in 0—4 hr urine except the amount of arauridine, it was slightly increased. Cyclocytidine was not so transformed to aracytidine *in vivo*, though antitumor activity of cyclocytidine was considered to be due to transformed aracytidine *in vitro*.[11] The cause of this contradictory result might be rapid excretion of cyclocytidine into urine. In other words, biological half-life in blood of cyclocytidine was too short to transform into aracytidine in blood stream, it is about 20 min[10] and the rate of transformation to aracytidine at 37° and pH 7.3 was only 10% per hour.[11]

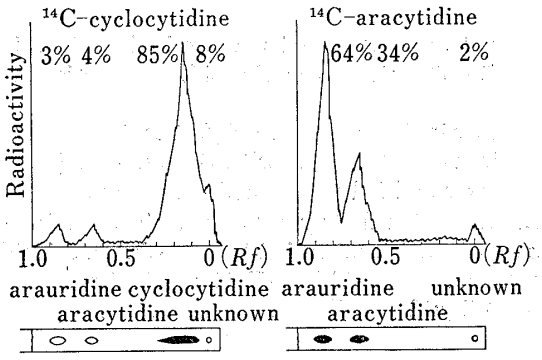


Fig. 2. Radiochromatograms of the Metabolites in Urine within Four Hours after Intravenous Injection of $^{14}$C-Cyclocytidine and $^{14}$C-Aracytidine

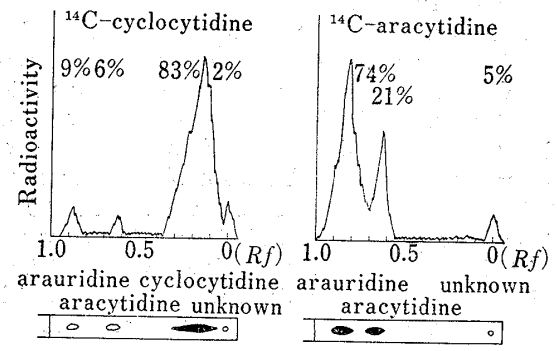


Fig. 3. Radiochromatograms of the Metabolites in Urine 4—24 Hours after Intravenous Injection of $^{14}$C-Cyclocytidine and $^{14}$C-Aracytidine

On the other hand, main part (64%) of the radioactive compounds in urine within 4 hours after intravenous administration of $^{14}$C-aracytidine as a reference compound was arauridine which was a deaminated product of aracytidine and 34% of total activity was intact aracytidine. Ratio of arauridine to aracytidine in 4—24 hr urine was somewhat increased. These results showed that cyclocytidine was resistant to cytidine deaminase *in vivo* similar to *in vitro* as reported previously,[12] because of slow transformation to aracytidine in tissues and rapid excretion into urine. Furthermore, the cause of high therapeutic index or lower toxicity of cyclocytidine *in vivo* is considered to be this slow transformation to aracytidine together with specific distribution in tissues of cyclocytidine.

## Rate of Excretion after Oral Administration

As shown in Fig. 4 and 5, about 77% and 79% of radioactivity were excreted from the body within 24 hours after oral administration of $^{14}$C-cyclocytidine and $^{14}$C-aracytidine, respectively. Ratio of excretion in feces, urine and expired air in the two compounds was different in each other though total excretion was similar in both. About a half of cyclocytidine was excreted in feces, whereas only one-tenth of aracytidine was excreted in feces. Since

11) A. Hoshi, M. Yoshida, F. Kanzawa, K. Kuretani, T. Kanai, and M. Ichino, *Chem. Pharm. Bull.* (Tokyo), **21**, 1446 (1973).
12) A. Hoshi, M. Iigo, M. Saneyoshi, and K. Kuretani, *Chem. Pharm. Bull.* (Tokyo), **21**, 1535 (1973).

aracytidine was considered to be degradated in gastrointestinal tract and excreted in expired air as $CO_2$,[13] excretion in expired air was determined. Only small amount of radioactivity was excreted in expired air after oral administration of $^{14}$C-cyclocytidine, however, one-fourth of radioactivity was excreted after $^{14}$C-aracytidine administration. Excreted amount of cyclocytidine in urine was about a half of that of aracytidine, it means that aracytidine is more absorbed from gastrointestinal tract than cyclocytidine. It is contrary to the result that the activity of cyclocytidine against L1210 cells *in vivo* is higher than that of aracytidine.[7]

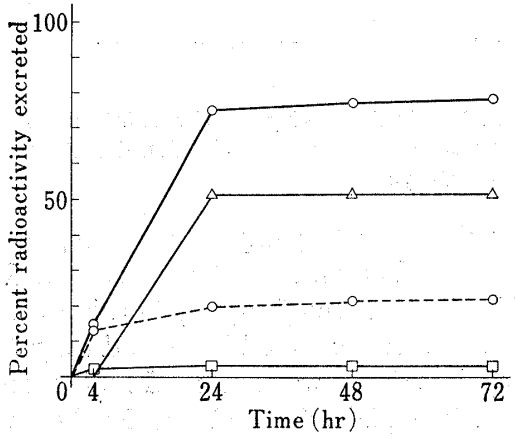


Fig. 4. Excretion of Radioactivity in Feces, Urine and expired $CO_2$ of Mice after Oral Administration of $^{14}$C-Cyclocytidine

—□—: expired $CO_2$ —△—: feces
---○---: urine —○—: total

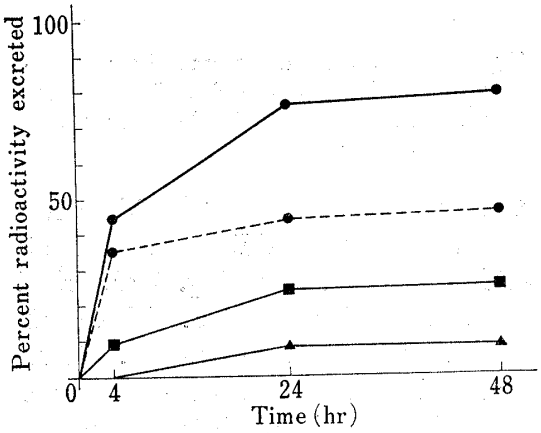


Fig. 5. Excretion of Radioactivity in Feces, Urine and expired $CO_2$ of Mice after Oral Administration of $^{14}$C-Aracytidine

—■—: expired $CO_2$ —▲—: feces
---●---: urine —●—: total

**Metabolites in Urine after Oral Administration**

Metabolites of cyclocytidine were detected in urine but not in feces. Radioactive compound in feces was intact cyclocytidine. As shown in Fig. 6, radioactive compounds in urine within 4 hours after oral administration of $^{14}$C-cyclocytidine were identified as cyclocytidine, aracytidine and arauridine similar to the case of intravenous administration. The compound at origin was not identified. Main part of radioactive compounds was cyclocytidine (89%) and small amounts of aracytidine and arauridine were detected in 0—4 hr urine. Ratio of arauridine to aracytidine in 4—24 hr urine was somewhat in creased and 75% of radioactive compounds was cyclocytidine itself. On the other hand, main part of radioactive compounds was arauridine after administration of $^{14}$C-aracytidine. Ratio of arauridine was 66% and 72% in 0—4 hr and 4—24 hr urine, respectively.

**Absorption of Cycytilocdine from Gastrointestinal Tract**

The absorption behavior of cyclocytidine in gastrointestinal tract was investigated. Each of the residual amounts in three parts (stomach, small intestine and caecum plus large intestine) of the tract was measured and the results obtained from 5 mice are shown in Fig. 7.

One-fifth of $^{14}$C-cyclocytidine was absorbed from stomach within 1 hour after oral administration. One hour after oral administration, main part of radioactivity existed in small intestine. Two hours after administration, radioactivity transfered from small intestine to large intestine including caecum, but radioactivity did not decrease during that time. It meant that cyclocytidine was scarcely absorbed from small intestine. Since two hours after administration, radioactivity in large intestine slowly decreased. It meant that cyclocytidine was slowly absorbed from large intestine. Almost all of radioactive compounds in gastro-

13) L.B. Mellett, S.M. ElDareer, and F.P. Chen, *Proc. Am. Ass. Cancer Res.*, **1971**, 68 (1971).

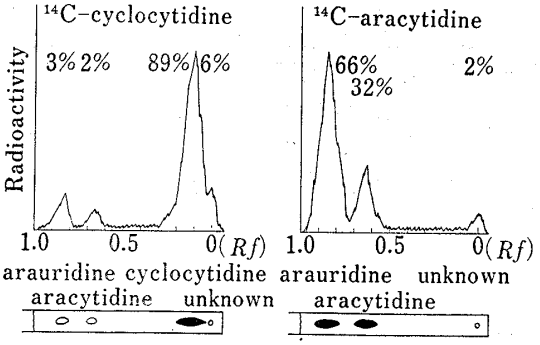


Fig. 6. Radiochromatograms of the Metabolites in Urine within Four Hours after Oral Administration of $^{14}$C-Cyclocytidine and $^{14}$C-Aracytidine

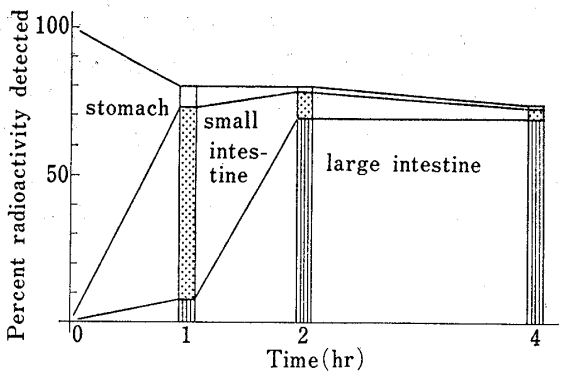


Fig. 7. Radioactivity in Gastrointestinal Tract Contents after Oral Administration of $^{14}$C-Cyclocytidine

intestinal contents was cyclocytidine itself as determined by thin-layer chromatography. It showed that cyclocytidine was more stable than aracytidine and was not transformed to aracytidine. As shown in Fig. 4, absorbed cyclocytidine was excreted rapidly in urine within 4 hours and slowly thereafter. Unabsorbed cyclocytidine was excreted in feces during 4 to 24 hours after oral administration. It meant that cyclocytidine was absorbed mainly from stomach and slightly from large intestine. These results explained the absorption behavior of cyclocytidine and low distribution in tissues after oral administration.[10]