[Chem. Pharm. Bull. 23(4) 725—728 (1975)]

UDC 615.31.033.076.9 : 547.963.32.09

Distribution of Cyclocytidine in Tissues[1]

Akio Hoshi, Masaaki Iigo, 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 June 5, 1974)

The distribution and changes of concentration of cyclocytidine in tissues with time were quantitatively examined after intravenous injection or oral administration. Localized distribution of the compound in tissues was confirmed quantitatively and retention of the compound in most of the tissues tested was found out. Whereas, aracytidine distributed uniformly in tissues and elimination rate of the compound from tissues was similar in each other. After oral administration, cyclocytidine distributed in various tissues at very low levels.

Cyclocytidine (2,2′-O-cyclocytidine) is markedly active against various mouse tumors[3] and acute leukemia clinically.[4] Distribution of cyclocytidine in organs and tissues was previously examined autoradiographically and it was found that the compound was localized in some organs, whereas aracytidine (1-β-D-arabinofuranosyl cytosine) was distributed uniformly in tissues.[5] In the present work, the distribution and changes of concentration of cyclocytidine in tissues as a function of time were quantitatively examined after intravenous injection or oral administration.

Materials and Method

Labeled Compounds——2-^{14}C-Cyclocytidine and 2-^{14}C-aracytidine were synthesized from 2-^{14}C-cytidine by the authors.[6]

Procedures——Female ddN mice weighing 20±1 g were used throughout the experiments. Radioactive compounds were dissolved in physiological saline and administered intravenously from the tail vein or orally by a stomach tube at the dose of 50 μCi (34 mg as cyclocytidine or 36 mg as aracytidine)/kg. After administration of the compound, mice were given diet and water *ad libitum*. Mice were anesthetized with ether and sacrificed 10, 30, 60 min and 24 hr after intravenous injection or 1, 2, 4, and 24 hr after oral administration. Blood samples were drawn from the vena cava. The tissue samples were excised after the animals were killed by bleeding from the carotid artery. One-tenth milliliter of whole blood or 100 mg each of tissues were dissolved in 2 ml of Soluene (Packard). Two animals were used in a group. Radioactivity was determined with a Tri-Carb Model 3320 liquid scintillation spectrophotometer using Toluene-PPO-dimethylPOPOP as the scintillator system after adjusted the pH of scintillator at 7.0 with AcOH.

1) This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education. 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.*

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Result

Distribution of Cyclocytidine after Intravenous Injection

As shown in Table I, the highest concentration of cyclocytidine was shown by kidney 10 min after injection and the concentration was as high as about 150 μg/g. The compound was distributed also in liver and salivary gland at higher concentration and the concentration of cyclocytidine was about 80 and 90 μg/g in liver and salivary gland, respectively. Concentration of the compound in other tissues except skeletal muscle was higher than that in blood at any time after 10 min. Cyclocytidine was accumulated in tissues at higher concentration than that in blood. However, concentration of the compound in skeletal muscle was one-half of that in blood.

Concentration of cyclocytidine in blood decreased as a function of time with a biological half-life of 20 min. Cyclocytidine in liver decreased with time in a similar rate as in blood. On the other hand, decrease of the compound was very slow in almost all of other tissues such as spleen.

Table I. Concentration of ^{14}C-Cyclocytidine in Tissues after Intravenous Injection

Tissue	Concentration in tissue[a] (μg/ml or g wet weight)			
	10 min	30 min	60 min	24 hr
Blood	10.0± 4.4	3.7± 0.1	1.9± 0.3	0.2±0.1
Liver	83.6± 1.6	32.6± 5.9	15.6± 1.6	0.7±0.2
Kidney	149.4±36.5	31.1±12.3	29.7±19.1	1.8±0.6
Spleen	10.4± 3.1	8.8± 1.6	8.9± 1.5	1.8±0.4
Lung	26.2± 1.5	23.0± 3.2	14.9± 1.8	0.8±0.1
Pancreas	22.3± 6.2	12.4± 4.4	16.3± 0.2	1.7±0.1
Myocardium	30.1± 6.0	34.9± 6.6	19.4± 2.1	3.6±0.4
Skeletal muscle	4.1± 0.1	2.5± 0.1	2.8± 0.1	0.5±0.1
Salivary gland	92.4±20.2	94.1±14.5	79.5± 0.6	14.3±0.2

a) Mean ±SD of two mice in a group. 34 mg (50 μCi)/kg of cyclocytidine was injected. Concentration was calculated as cyclocytidiine.

Distribution of Aracytidine after Intravenous Injection

Distribution of aracytidine was examined as a reference. In contrast to the case of cyclocytidine, concentration of aracytidine in tissues was not so different among the tissues. Biological half-life of the compound in blood were about 30 min. Elimination rate of the compound from tissues was also similar in each other.

Distribution of Cyclocytidine after Oral Administration

Cyclocytidine was active against L1210 leukemia by oral treatment but the potency was weaker than that by intraperitoneal treatment.[7] Concentration of cyclocytidine in tissues after oral treatment was examined to determine whether cyclocytidine distributes in high concentration or not. After oral administration, the compound distributed in various tissues at very low levels, whose concentration in tissues except skeletal muscle was higher than that in blood at any time after 1 hr and this low level of the compound retained for 4 hr or more. The concentration in salivary gland was very high at any time.

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

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Table II. Concentration of ^{14}C-Aracytidine in Tissues after Intravenous Injection

Tissue	Concentration in tissue[a] (μg/ml or g wet weight) 10 min	30 min	60 min	24 hr
Blood	26.8±4.3	17.3± 1.7	8.6±0.2	0.1±0.1
Liver	30.9±0.4	20.5± 2.1	10.7±1.0	1.2±0.1
Kianey	58.6±3.2	45.3±14.1	22.2±3.7	1.2±0.2
Spleen	46.3±1.8	37.3± 6.2	19.5±5.1	1.2±0.1
Lung	35.8±0.1	23.1± 1.8	11.6±0.4	0.7±0.1
Pancreas	31.1±0.7	21.0± 2.8	11.7±0.1	1.1±0.1
Myocardium	40.6±0.2	24.9± 1.5	11.4±0.3	0.4±0.1
Skeletal muscle	33.1±0.7	21.3± 2.7	9.9±0.4	0.2±0.1
Salivary gland	37.2±2.0	22.4± 0.1	13.0±0.1	1.0±0.3

a) Mean ± SD of two mice in a group. 36 mg (50 μCi/kg of aracytidine was injected. Concentration was calculated as aracytidine.

Table III. Concentration of ^{14}C-Cyclocytidine in Tissues after Oral Administration

Tissue	Concentration in tissue[a] (μg/ml or g wet weight) 1 hr	2 hr	4 hr	24 hr
Blood	0.4±0.1	0.3±0.1	0.4±0.1	<0.1
Liver	4.4±1.0	2.5±0.2	1.4±0.8	0.2±0.1
Kidney	4.9±0.4	1.9±0.1	1.1±0.6	0.1±0.1
Spleen	1.0±0.3	1.3±0.1	1.1±0.3	0.3±0.1
Lung	2.0±0.2	2.3±0.1	2.1±0.1	0.1±0.1
Pancreas	1.3±0.3	2.3±0.3	2.5±0.8	0.2±0.1
Myocardium	2.9±0.4	4.4±1.3	2.9±0.5	0.4±0.1
Skeletal muscle	0.4±0.1	0.4±0.1	0.4±0.1	<0.1
Salivary gland	8.6±2.5	13.0±4.7	11.3±2.3	2.5±0.5

a) Mean ± SD of two mice in a group. 34 mg (50 μCi)/kg of cyclocytidine was administered. Concentration was calculated as cyclocytidine.

Discussion

Cyclocytidine was found as a water soluble "transport form" of aracytidine in chemical level.[8] Furthermore, cyclocytidine primarily inhibited the incorporation of thymidine into DNA in a manner similar to aracytidine presumably after transformation to the latter compound *in vitro*.[8] Significant difference between cyclocytidine and aracytidine was observed in toxicity especially in cumulative toxicity. The former was less toxic than the latter.[3] In previous report,[5] the distribution of cyclocytidine was investigated by means of whole-body autoradiographic technique and a significant difference was found in the distribution pattern of radioactivity between cyclocytidine and other cytidine analogues such as aracytidine.

The present result revealed that the elimination of cyclocytidine from tissues was very slow, in other words, the compound was retained in tissues for a long period. It was an interesting fact that cyclocytidine was retained especially in spleen though the concentration was low, because this organ was a hematogenic tissue and cyclocytidine have a marked anti-leukemic activity. Retention or accumulation in salivary gland of the compound may be the cause of parotid pain which is one of the side effects of the compound. Concentration

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of aracytidine in tissues 10 min after injection was similar among tissues tested. Uniform distribution of the compound in tissues observed in whole-body autoradiography was confirmed quantitatively by the present experiments.

Cyclocytidine was active against L1210 leukemia when administered orally, but the potency was weaker than that by intraperitoneal injection.[7] Concentration of cyclocytidine in tissues was therefore examined by quantitative analysis. Concentration of the compound in tissues after oral administration was very low, however, the compound was still detected in tissues 4 hours or more after administration at over 1 μg/g tissue. The cause of insufficient effectiveness of cyclocytidine by oral treatment was considered to be due to these lower concentration in tissues. In conclusion, distribution of cyclocytidine in tissues was found to be more localized than that of aracytidine and elimination of the former compound was slower than that of the latter in most of all tissues.

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