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Specific Inhibition of DNA Synthesis by Cyclocytidine

Antitumor activity of cyclocytidine was first found in the authors laboratory using various mouse tumors including L1210 leukemia and solid tumors.[1,2] Cyclocytidine[3] (2,2'-O-cyclocytidine hydrochloride; 2,2'-anhydro-1-β-D-arabinofuranosylcytosine hydrochloride) is similar to aracytidine (1-β-D-arabinofuranosylcytosine hydrochloride) and is chemically one of the cytidine analogs. Unity and diversity in the mechanism of actions of cyclocytidine and aracytidine were examined in cultured cells.

L5178Y leukemia cells were cultured in RPMI 1640 medium (10% calf serum added) in a CO_2 incubator at 37°. Antiutmor activity was determined by the ratio of cell number in treated and control groups (T/C%) after 48 hr incubation at various concentrations of the compound, and IC_{50} (50% inhibiting concentration) was calculated. DNA, RNA, and protein synthesis were determined by the respective incorporation of ^{14}C-labeled thymidine, uridine, and L-leucine into the cold trichloroacetic acid insoluble fraction of the cells after incubation for 30 min.

Cyclocytidine inhibited the growth of the leukemia *in vitro* and its IC_{50} was 0.041 μg/ml, wheras that for aracytidine was 0.023 μg/ml. Potency of cyclocytidine *in vitro* was one-half of that of aracytidine.

Mechanism of action of the compound was further examined at the level of biosynthesis in intact cells. The compound specifically inhibited thymidine incorporation into the cold trichloroacetic acid insoluble fraction but not that of uridine or L-leucine as shown in Fig. 1. IC_{50} for cyclocytidine in inhibition of thymidine incorporation was 110 μg/ml instead of 1.1 μg/ml for aracytidine. Cyclocytidine itself was considered to have no activity against DNA biosynthesis of the leukemia according to the difference in these two values.

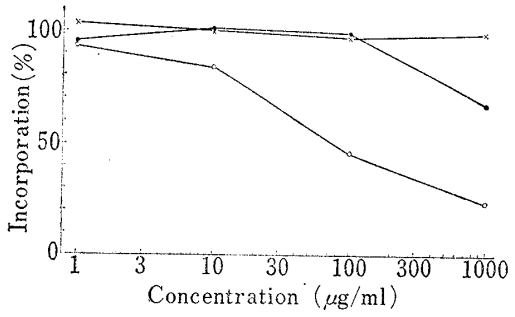

Fig. 1. Effect of Cyclocytidine on Incorporation of ^{14}C-Labeled Precurosrs

—○—: ^{14}C-thymidine
—●—: ^{14}C-uridine
—×—: ^{14}C-L-leucine

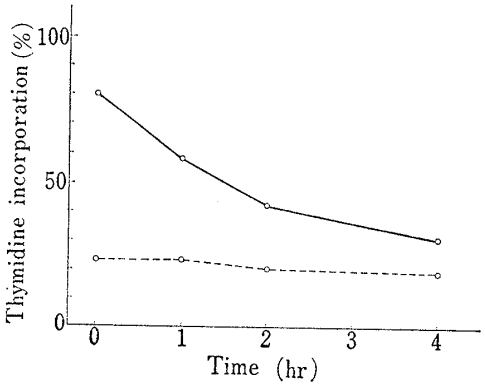

Fig. 2. Effect of Cyclocytidine on Thymidine Incorporation withTime

—○—: cyclocytidine (10 μg/ml)
···○···: aracytidine (10 μg/ml)

As for the mechanism of action of cyclocytidine, following three possibilities are suggested; (a) cyclocytidine itself acts similarly to aracytidine, (b) cyclocytidine acts after transformation to aracytidine, or (c) both of them. Since the possibility (b) seems to be the most reasonable from the above results, further examinations were made.

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As shown in Fig. 2, the activity of cyclocytidine dissolved in RPMI 1640 medium increased with time and the incorporation of thymidine into DNA for 30 min therefore decreased, while the activity of aracytidine was not affected under the same conditions. Metabolites of cyclocytidine after incubation for 4 hr without leukemia cells were further examined by thin-layer chromatography and aracytidine was detected as the sole metabolite of the compound. Therefore, the decrease in thymidine incorporation was considered to be due to the "transformed" aracytidine. Transforming ratio of cyclocytidine to aracytidine after standing for 4 hr was about 50% when calculated by the dose-response line for aracytidine.

It is concluded that cyclocytidine shows a marked antitumor activity *in vitro* as well as *in vivo* through the specific depression of DNA biosynthesis similar to aracytidine. This inhibition seems to be due to aracytidine "transformed" from cyclocytidine. In other words, cyclocytidine is considered to be a "transport form" of aracytidine with low toxicity.

Pharmacology Division,
National Cancer Center Research Institute
Chuo-ku, Tokyo

Akio Hoshi
Mitsuzi Yoshida
Fumihiko Kanzawa
Kazuo Kuretani

Research Laboratory, Division of
Fermentation and Chemical
Products, Kohjin Co., Ltd.
Saiki, Oita

Tadashi Kanai
Motonobu Ichino

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Synthesis of *dl*-Gingerol

Sufficient evidence[1,2a] has been reported on the structures of gingerol homologues[2] (I), pungent principles isolated from the root of *Zingiber officinale* Roscoe. No synthetic work has appeared in the literature in spite of their widespread uses in medicine and food industries. We describe here the first synthesis of racemate of gingerol (I, $n=4$), a major component[3] in the homologues.

Attempted one-step synthesis of it, the aldol condensation of zingerone (II) with caproic aldehyde in the presence of alkali such as KOH or K_2CO_3 under various conditions, however, was almost unsuccessful giving a number of undesirable products, recovered II, selfcondensation product of II, shogaol[4] (III) and others, along with a slight amount of *dl*-gingerol. No satisfactory method for the separation of gingerol from the mixture with chromatography could be developed due to the unavoidable decompositions of it to II on alumina and to III

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