STRUCTURE-ACTIVITY RELATIONSHIPS OF CADEGUOMYCIN ANALOGS

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The relationship between the activity and the chemical structure of cadeguomycin (CDM, 7-carboxy-7-deazaguanosine) was studied with six analogs of CDM. Both activities of CDM, enhancing the incorporation of [^{3}H]thymidine in K562 cells and potentiating the cytotoxicity of cytosine arabinoside for K562 cells, were significantly augmented by the replacement of the 7-carboxyl group with cyano (CDM-CN) or formyl (CDM-CHO), but they were not changed by the replacement with methyl. The activities were almost completely diminished by the replacement of ribose with arabinose, but the simultaneous replacement of carboxyl and ribose with formyl and arabinose showed higher activities than those of CDM. The replacement of 7-carboxy-7-deazaguanine with 7-carboxy-7-deazainosine markedly weakened the activity. CDM-CN and CDM-CHO at 0.2 μg/ml significantly potentiated the activity of cytosine arabinoside against MOLT-3 cells but CDM at 1 μg/ml did not. These results indicate that the ribose and guanine moieties in the CDM molecule are very important for its activity. Also replacing the carboxyl group at the C-7 position with cyano or formyl group is a useful way to strengthen the CDM activity. These compounds would effectively potentiate cytosine arabinoside against various kinds of tumor cells which CDM could not do.

Cadeguomycin (CDM) is a nucleoside antibiotic which was discovered because of its unique property of enhancing the incorporation of pyrimidine nucleosides, such as [^{3}H]thymidine, into K562 human myelogenous leukemia cells[1~3]. In mice, CDM retards the growth of sc solid IMC carcinoma and the pulmonary metastasis of Lewis lung carcinoma[4]. It strongly potentiates the cytotoxicity of 1-β-D-arabinofuranosylcytosine (ara-C)[5] and other cytotoxic cytosine analogs[6] against K562 cells. We have studied the structure-activity relationship of CDM analogs in the activities of enhancing the incorporation of [^{3}H]thymidine into K562 cells and the potentiation of ara-C against K562 and MOLT-3 cells, and we obtained analogs which have much stronger activity than CDM.

Materials and Methods

Chemicals

CDM (**1**) was prepared as described previously[2]. Several analogs (**2**~**6**) of CDM were synthesized from the key intermediate, 3,4-dihydro-3-methoxymethyl-5-methyl-2-methylthio-7-(2,3-*O*-isopropylidene-5-*O*-triphenylmethyl-β-D-ribofuranosyl)-7*H*-pyrrolo-[2,3-*d*]pyrimidin-4-one[7~9], except one (**7**) which was obtained as a degradative component of CDM. The chemical structures of these compounds are il-

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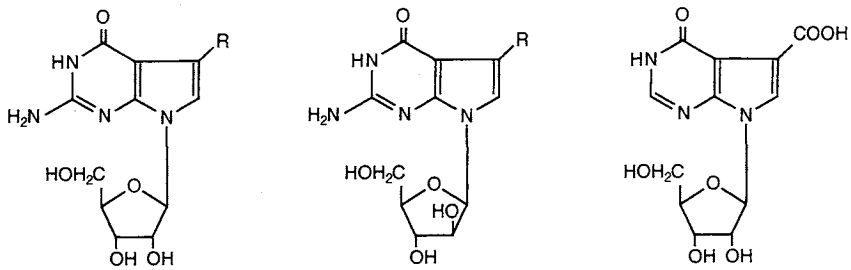
Fig. 1. Chemical structures of CDM analogs.



1 R=COOH
2 R=CH_3
3 R=CHO
4 R=CN

5 R=COOH
6 R=CHO

7

lustrated in Fig. 1. The structure of CDM (7-carboxy-7-deazaguanosine, **1**) was modified as follows: the 7-carboxyl group was replaced with formyl (**3** and **6**), cyano (**4**) or methyl (**2**), and the ribose was replaced with arabinose (**5** and **6**) and guanosine with inosine (**7**). The CDM and its analogs were dissolved in phosphate-buffered saline (PBS) and sterilized by Millipore filtration. Ara-C was purchased from Yamasa Shoyu Co., Tokyo and 5-fluorodeoxycytidine (5-fluoroCdR) and 5-azadeoxycytidine (5-azaCdR) were from Sigma Chemical Company, St. Louis, MO. *Methyl*-[^{3}H]thymidine (25 Ci/mmol) was a product of Amersham Japan.

Cells

K562 cells (human chronic myelogenous leukemia)[10] and MOLT-3 cells (human acute lymphoblastic leukemia)[11] were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum in a humidified atmosphere containing 5% CO_2 at 37°C.

Determination of [^{3}H]Thymidine Incorporation

K562 cells (5×10^4/ml) were cultured in a 96-well microplate for 18 hours in the presence of various concentrations of CDM or its analogs (total 0.2 ml/well), then incubated with 2 μCi/ml of [^{3}H]thymidine for 2 hours. The cells were collected on a glass fiber filter and washed first with PBS, then with cold 5% TCA, using a cell harvester (Abe Kagaku). The radioactivity on the filter was determined with a liquid scintillation counter. The ratio of [^{3}H]thymidine incorporation in the presence and absence of CDM or its analogs was taken as the stimulation index.

Potentiation of Cytotoxicity of Ara-C or Other Pyrimidine Nucleosides

K562 or MOLT-3 cells (2×10^4/ml) were cultured in a 24-well plate (2 ml of cell suspension/well) with various concentrations of ara-C, 5-fluoroCdR or 5-azaCdR in the presence or absence of CDM analogs. The viable cell number was determined by a trypan blue dye exclusion method after 5 days. CDM and its analogs alone did not affect the growth of the cells at the concentrations used.

In these experiments, the mean values of triplicate asays were calculated. The variation was less than 10%.

Results

Effects on [^{3}H]Thymidine Incorporation in K562 Cells

CDM enhanced the incorporation of [^{3}H]thymidine into K562 cells approximately 2-, 4-, and 7-fold at a concentration of 0.04, 0.2, and 1 μg/ml, respectively, after 18-hour preincubation with the cells. Most of the CDM analogs showed different activity from CDM. **5** did not show enhancement of [^{3}H]thymidine incorporation at the concentrations tested, indicating the importance of a ribose moiety for this activity.

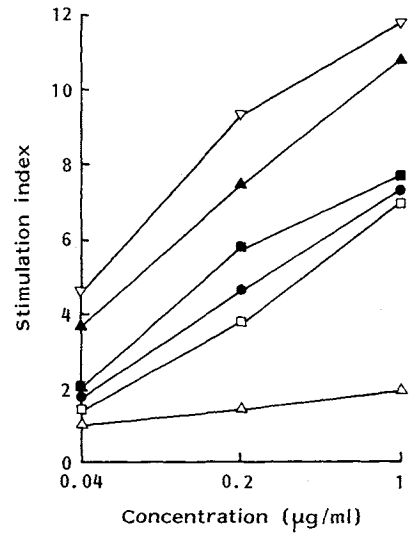
The replacement of the carboxyl group at the C-7 position with $-CH_3$ (**2**) did not affect activity, but its replacement with –CHO (**3**) or –CN (**4**) greatly strengthened it, and the activity of the latter was slightly higher than the former. Although **5** lost activity, **6** showed higher activity than CDM (Fig. 2).

Potentiation of the Cytotoxicity of Ara-C in K562 Cells by CDM Analogs

The effect of ara-C on the growth of K562 cells was studied in the absence or presence of 0.2 μg/ml of CDM or its analogs, which did not significantly affect growth. As illustrated in Fig. 3, the cytotoxicity of ara-C was markedly enhanced by CDM at 0.2 μg/ml. The IC_{50} was 270 nM in the absence of CDM and 30 nM in the presence of CDM; the degree of potentiation was 9.0. **5** and **7** did not potentiate ara-C cytotoxicity at 0.2 μg/ml. **7** at 5 μg/ml potentiated it 3.1-fold (data not shown). Again, **2** showed activity similar to CDM, and **3** and **4** exhibited the highest potentiating activity among the CDM analogs; the potentiation ratio was 29.3 and 31.8, respectively. **6** potentiated ara-C cytotoxicity 15.0-fold, which was higher than that of CDM.

Fig. 2. Stimulation by CDM analogs of [^{3}H]thymidine incorporation in K562 cells after 18 hours of pre-incubation.

● **1**, □ **2**, ▲ **3**, ▽ **4**, △ **5**, ■ **6**.



Comparison of the Potentiating Activity for Pyrimidine Nucleosides between CDM and 3

As previously reported[6], CDM potentiated the cytotoxicity of not only ara-C but also other cytosine nucleoside analogs, although the highest potentiation was in ara-C cytotoxicity. The potentiating activity for cytosine nucleoside analogs was

Fig. 3. Effect of CDM analogs on ara-C cytotoxicity for K562 cells.

The cells were cultured for 5 days with various concentrations of ara-C in the absence or presence of 0.2 μg/ml of CDM or its analogs. ○ Control, ● **1**, □ **2**, ▲ **3**, ▽ **4**, △ **5**, ■ **6**, ▼ **7**.

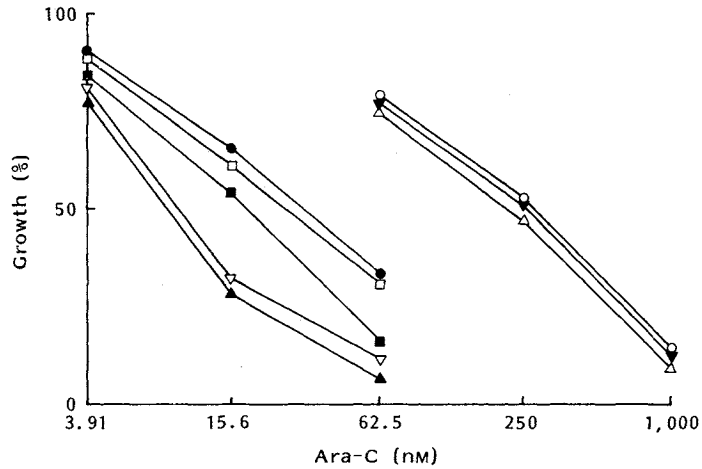


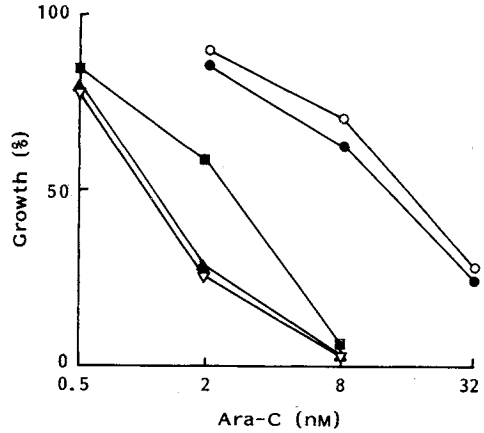
Table 1. Potentiation of cytotoxic cytosine nucleoside analogs by CDM (**1**) and **3** for K562 cells.

Nucleosides	IC_{50} of nucleosides (μM)		
	None	**1** (1 μg/ml)	**3** (0.2 μg/ml)
Ara-C	0.27	0.0073 (37.0)[a]	0.0085 (31.8)
5-FluoroCdR	6.2	0.61 (10.2)	0.68 (9.1)
5-AzaCdR	0.10	0.019 (5.3)	0.012 (8.3)

[a] Potentiation ratio.

Fig. 4. Effect of CDM analogs on ara-C cytotoxicity for MOLT-3 cells.

The experiment was carried out as described in Fig. 3, except that the concentration of CDM was 1 μg/ml. ○ Control, ● **1**, ▲ **3**, ▽ **4**, ■ **6**.



compared between CDM and **3** which was the highest potentiator for ara-C among the CDM analogs tested. In this experiment, CDM was used at 1 μg/ml, since there was no potentiation for 5-azaCdR at the concentration of 0.2 μg/ml. As summarized in Table 1, **3** at 0.2 μg/ml potentiated the activity of ara-C, 5-fluoroCdR, and 5-azaCdR to an extent similar to CDM at 1 μg/ml, and like CDM, its highest potentiation was against ara-C. These results indicate that the potentiating activity of **3** is approximately 5 times higher than that of CDM.

Potentiation of Ara-C in MOLT-3 Cells by CDM Analogs

Potentiation of ara-C cytotoxicity and the enhancement of pyrimidine nucleoside incorporation by CDM were restricted to specific cell lines such as K562 and YAC-1 cells (murine T-cell lymphoma induced by Molony leukemic virus) and there were no effects on MOLT-3, mouse FM3A, L5178Y or L1210 cells[3,4]. Because a few CDM analogs exerted stronger potentiating activity for ara-C than CDM, we tested whether they potentiated ara-C in MOLT-3 cells, which CDM failed to potentiate. As illustrated in Fig. 4, CDM at 1 μg/ml did not change the activity of ara-C, but **6**, **3** and **4** at 0.2 μg/ml significantly potentiated it; the potentiation ratios were 6.5, 12.5 and 13.6, respectively. These results indicate that **3** and **4** are especially effective potentiators of the cytotoxicity of ara-C and other cytotoxic pyrimidine nucleosides in various kinds of tumor cells.

Discussion

CDM, a 7-deazaguanosine antibiotic, was produced by a strain of *Streptomyces hygroscopicus* which was also producing tubercidin, a 7-deazaadenosine antibiotic. CDM and tubercidin had quite different effects. CDM was discovered by the unique activity of stimulation of the incorporation of [^{3}H]thymidine into K562 cells[1], whereas tubercidin was discovered by screens for antibacterial and antitumor activities[1,2]. Therefore, we studied the relationship between the structure and activity of CDM analogs.

The replacement of the carboxyl group at C-7 of the CDM with a formyl or cyano group greatly strengthened the activity, indicating that a strong electron-withdrawing group at this position is preferable for its activity. The change of the ribose moiety of CDM to arabinose or the change of 7-deazaguanine to 7-deazainosine greatly decreased the activity. As previously reported[3], the effect of CDM on the enhancement of [^{3}H]thymidine uptake in K562 cells was markedly reversed by adding a high concentration of guanosine or deoxyguanosine, but not affected by adding adenosine or deoxyadenosine. This suggests that the replacement of the ribose in CDM with deoxyribose would not affect activity but the replacement of the guanine moiety of CDM with an adenine moiety would lose the CDM activity. From the data in

this and the previous paper[3], the structure-activity relationship of CDM analogs could be summarized as follows: the group at C-7: CHO=CN>COOH=CH_3; sugar: ribose=deoxyribose≫arabinose; base: guanine≫adenine=hypoxanthine.

7-Deazaadenosine antibiotics such as tubercidin, toyocamycin and sangivamycin strongly inhibit nucleic acid synthesis and tumor cell growth, and a 7-deazaguanosine skeleton is suggested to be an intermediate in the biosynthesis of tubercidin[13]. Like **4**, toyocamycin also has a cyano group at the C-7 position, and the structural difference between **4** and toyocamycin exists only in the adenine and guanine moiety. However, their activities are quite different, clearly indicating the importance of the base for each activity. Among the 7-deazaadenosine antibiotics mentioned above, only sangivamycin, which has a carbamoyl group at C-7, showed a strong inhibitory activity on protein kinase C[14,15]. This also indicates the importance of a group at C-7. **7** has already been described in the literature as a chemical degradation product of toyocamycin[16,17]. The Q base (queuine), another 7-deoxyguanine derivative, has been discovered as a unique base in tRNA of prokaryotes and eukaryotes[18]. NARUTO *et al.* reported kanagawamicin[19], another 7-deazaguanosine antibiotic, which has a modified sugar in the form of 2-amino-2-deoxyarabinofuranose and shows antitumor activity and weak antibacterial activity against Gram-negative bacteria. However, an activity like CDM has not been described in these nucleosides.

Potentiation of ara-C by CDM was not significant in MOLT-3 cells, as described in this paper. However, recently we have isolated ara-C-resistant MOLT-3 cells by a stepwise increase in ara-C concentration in the culture and found that CDM strongly potentiates ara-C activity in these cells and overcomes the resistance. This cell line shows a higher activity than the parental cell line of dCMP deaminase, which inactivates ara-C by converting ara-CMP to ara-UMP. The activity of dCMP deaminase in K562 cells is higher than that in other cells in which there is no potentiation of ara-C by CDM. These results suggest that CDM blocks the activity of dCMP deaminase, resulting in an increase in the amounts of araCTP, the final active form of ara-C, in the cells[5] and the difference in the amount of dCMP deaminase may explain the cell specificity of CDM for ara-C potentiation. Details will be presented elsewhere. The affinity of the CDM analogs or thier metabolites with dCMP deaminase would be different from each other and this would be the cause of the difference in the activity of ara-C potentiation.

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