# EFFECTS OF CADEGUOMYCIN ON CYTOTOXICITY OF CYTOSINE ARABINOSIDE AND OTHER PYRIMIDINE NUCLEOSIDE ANALOGS; A COMPARATIVE STUDY

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Cadeguomycin, which has been isolated from *Streptomyces hygroscopicus*, is a nucleoside antibiotic with the unique property of enhancing pyrimidine nucleoside incorporation into K562 human myelogenous leukemia cells[1~3]. Although the antibiotic exhibits weak growth inhibitory activity for cultured K562 cells ($IC_{50}$, 50% growth-inhibitory concentration is more than 25 $\mu$g/ml), it strongly potentiates cytotoxicity of 1-$\beta$-D-arabinofuranosylcytosine (Ara-C) against K562 cells by increasing cellular uptake of Ara-C and formation of Ara-C nucleotides[4]. In this communication, we examined the potentiation activity of cadeguomycin on cytotoxicity of other pyrimidine nucleoside analogs which exhibit antitumor activity.

As seen in Fig. 1, the cytotoxicity of 5-fluorodeoxycytidine (FCdR) was markedly enhanced by addition of 1 $\mu$g/ml cadeguomycin, a level which did not affect the growth of K562 cells by itself. The $IC_{50}$ of FCdR was 6.2 $\mu$M in the absence of cadeguomycin, whereas it was 0.61 $\mu$M in the presence of cadeguomycin; the degree of potentiation was 10.2. The effects of cadeguomycin on the cytotoxicity of other pyrimidine nucleoside and base analogs against K562 cells were determined and the $IC_{50}$ values in the presence or absence of cadeguomycin are presented in Table 1. Activity of Ara-C was greatly potentiated by cadeguomycin as previously reported[4] and those of 5-azadeoxycytidine and 5-azacytidine were also enhanced by the antibiotic at 1 $\mu$g/ml, although the potentiation ratios were less than that of FCdR. On the contrary, 5-fluorodeoxyuridine (FUdR), 3-deazauridine and 5-fluorouracil (5-FU) were not potentiated, suggesting that the potentiation activity of cadeguomycin is restricted to cytidine and deoxycytidine analogs.

Recently, we have found that the dCMP deaminase activity in K562 cells was greatly decreased by cadeguomycin treatment. This enzyme converts dCMP to dUMP and also inactivates Ara-C by converting Ara-CMP to Ara-UMP. Thus, the potentiation of the cytotoxicity of cytidine and deoxycytidine analogs by

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Fig. 1. Enhancement of cytotoxicy of 5-fluorodeoxycytidine against K562 cells by cadeguomycin.

Two ml of cell suspension ($2\times10^4$/ml) were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum in a 24-well plastic plate at 37°C with various concentrations of 5-fluorodeoxycytidine in the presence (●) or absence (○) of 1 $\mu$g/ml cadeguomycin. The viable cells were determined after 5 days, and the average of triplicate determinations is presented.

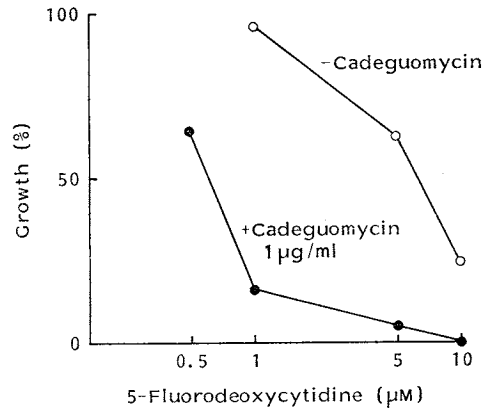


Table 1. Effects of cadeguomycin on cytotoxicity of pyrimidine base and nucleoside analogs for growth of K562 cells.

| Analogs | $IC_{50}$ of analogs ($\mu$M) | | Potentiation ratio |
|---|---|---|---|
| | Cadeguomycin 0 $\mu$g/ml | Cadeguomycin 1 $\mu$g/ml | |
| Ara-C | 0.35 | 0.0062 | 56.4 |
| 5-Fluoro CdR | 6.2 | 0.61 | 10.2 |
| 5-Aza CdR | 0.10 | 0.019 | 5.3 |
| 5-Aza CR | 1.35 | 0.45 | 3.0 |
| 5-Fluoro UdR | 1.9 | 1.6 | 1.2 |
| 3-Deaza UR | 0.25 | 0.35 | 0.71 |
| 5-Fluoro Ura | 8.2 | 15 | 0.55 |

CdR: Deoxycytidine, CR: cytidine, UdR: deoxyuridine, UR: uridine, Ura: uracil.

cadeguomycin seems to be due to inhibition of inactivation of these analogs. Ara-C might be the best substrate for the enzyme among the cytidine and deoxycytidine analogs tested here. FCdR is more active than 5-FU and FUdR against certain experimental tumors[5], and is believed to exert its cytotoxicity mainly by inhibiting thymidylate synthetase after conversion to FdUMP[6], like 5-FU and FUdR. However, our results that cadeguomycin potentiates the activity of FCdR and not 5-FU and FUdR, suggest that FCdR exerts its cytotoxicity by formation of FCdR nucleotides rather than conversion to uridine base by deamination, and they support the concept that FCdR is incorporated into DNA after phosphorylated to FdCTP[7]. Cadeguomycin is shown to be useful for potentiation of the cytotoxicity of not only Ara-C but also other antitumor substances of cytidine and deoxycytidine analog *in vitro*. We are now trying combination therapy of cadeguomycin and Ara-C or these analogs in experimental tumors *in vivo*.

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