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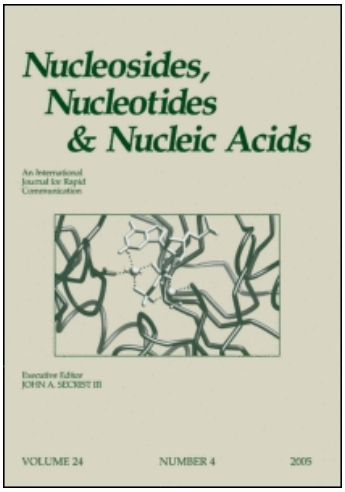

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
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Development Of An Antitumor Adenosine Analog, 3''-Ethynyladenosine

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To cite this Article Endo, Yoshio , Obata, Tohru , Nomura, Makoto , Fukushima, Masakazu , Yamada, Yuji , Matsuda, Akira and Sasaki, Takuma(2007) 'Development Of An Antitumor Adenosine Analog, 3''-Ethynyladenosine', Nucleosides, Nucleotides and Nucleic Acids, 26: 6, 691 — 694
To link to this Article: DOI: 10.1080/15257770701490654
URL: http://dx.doi.org/10.1080/15257770701490654

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Nucleosides, Nucleotides, and Nucleic Acids, 26:691–694, 2007
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ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701490654

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DEVELOPMENT OF AN ANTITUMOR ADENOSINE ANALOG, 3′-ETHYNYLADENOSINE

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□ *3′-ethynyladenosine (EAdo) was an adenosine analog with potent antitumor activity against various human tumor cells* in vitro. *However, EAdo was enzymatically inactivated by adenosine deaminase (ADA)* in vitro *and* in vivo. *Therefore, we synthesized two ADA-resistant EAdo derivatives (2-F-EAdo and EAdo-5′-monophosphate, EAMP) and examined their antitumor activities.*

Keywords 3′-ethynyladenosine (EAdo); antitumor mechanism; adenosine kinase (AK); adenosine deaminase (ADA)

INTRODUCTION

Several 2′-deoxyadenosine analogs such as cladribine, fludarabine, and clofarabine are used in the treatment of lymphoid malignancies.[1] These nucleosides are metabolically activated by phosphorylation enzymes including deoxycytidine kinase (dCK), thereby inhibiting DNA polymerases and ribonucleotide reductase, while also showing resistance to adenosine deaminase (ADA).[2]

With the aim of developing more potent antitumor nucleosides than 2′-deoxyadenosine analogs reported, 3′-ethynyladenosine (EAdo) was designed as an RNA synthesis inhibitor (Figure 1). EAdo was phosphorylated

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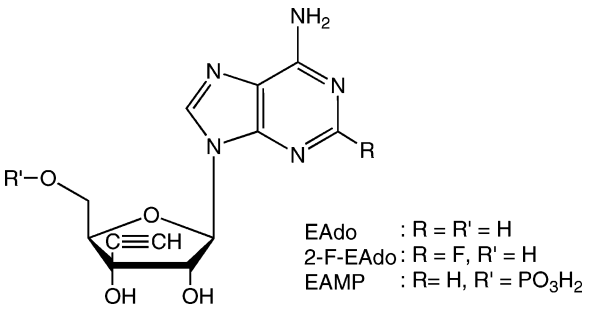

FIGURE 1 Structure of EAdo analogs.

by adenosine kinase (AK) and effectively inhibited RNA biosynthesis in tumor cells. EAdo, however, was ineffective on sarcoma-180 solid type tumor *in vivo*. Therefore, we synthesized two ADA-resistant EAdo derivatives (2-F-EAdo and EAdo-5′-monophosphate, EAMP). Although 2-F-EAdo was highly resistant to ADA, its antitumor activity was less than that of EAdo. On the other hand, EAMP showed the antitumor effect *in vivo* on sarcoma-180 solid type tumor.

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Anti-Proliferation Activity

In the *in vitro* MTT assay (72-hr continuous exposure), EAdo showed the anti-proliferation activity in the range of 0.05 μM to 1.5 μM against various human tumor cell lines including gastric, lung, and colon cancers (Table 1). RNA synthesis in gastric cancer MKN-45 cells effectively decreased by the treatment of EAdo for 1 hr. Thus, the main antitumor action of EAdo would be attributed by the RNA biosynthesis inhibition in tumor cells. The anti-proliferation activity of EAdo was lower than that of 3′-ethynylcytidine (ECyd),[3] but the activity was sufficient to induce apoptosis in MKN-45 cells.

TABLE 1 Anti-proliferative activity of EAdo

Cell line	IC_{50} value (μM)
MKN-45	0.4
NUGC-3	0.8
MKN-28	1.5
TMK-1	1.1
A549	0.9
Colo320	0.05
SW480	0.2
HT-29	0.2
HT-1080	0.5

Mechanisms of Antitumor Action and Drug Resistance

To elucidate the metabolic pathway of EAdo, non-nucleoside AK inhibitor ABT-702 at non-cytotoxic concentrations was added in the medium with EAdo in the MTT assay. ABT-702 effectively decreased the anti-proliferative activity of EAdo against the human gastric cancer cell line, NUGC-3. Furthermore, the EAdo-resistant cell line, NUGC-3/EAdo was sensitive to gemcitabine and ECyd which are phosphorylated by dCK and uridine/cytidine kinase 2, respectively.[4] The cellular AK activity in NUGC-3/EAdo was only 1/100 as compared with that of parental NUGC-3. The loss of AK activity was caused by decreased and aberrant AK mRNA expression with a 151-bp deletion which corresponded to exons 8 and 9. This deletion caused a non-sense mutation by the frameshift. Moreover, NUGC-3/EAdo-6xHis-hAK cells stably expressing human AK restored the sensitivity to EAdo. These data obviously indicate that AK is essential for EAdo phosphorylation and also is a main target for acquired resistance.

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Deamination of EAdo by ADA

In the *in vivo* assay using murine lymphoid leukemia, the administration of EAdo at 8 mg/kg significantly prolonged the survival days of the mice bearing P388 ascites tumors (T/C, 142%). In contrast, EAdo was ineffective on sarcoma-180 solid type tumor *in vivo*. Therefore, we considered that EAdo would be enzymatically inactivated by ADA. ADA from bovine spleen completely metabolized EAdo to 3′-ethynylinosine.

ADA-Resistant EAdo Derivatives

To improve the *in vivo* antitumor efficacy of EAdo, we first synthesized 2-F-EAdo as a ADA-resistant EAdo derivative. Although 2-F-EAdo showed a high resistance to ADA, the anti-proliferative activity of 2-F-EAdo was lower than that of EAdo. The antitumor activity of 2-F-EAdo appeared to strongly depend on the cellular AK activity, because colorectal cancer SW620 with high sensitivity to 2-F-EAdo had markedly increased AK activity as compared with that of MNNG/HOS with lower sensitivity. Next, we synthesized EAMP as a prodrug type of EAdo and examined its antitumor activity *in vivo* on sarcoma-180 solid type tumor. Sarcoma-180 cells (5×10^6) were inoculated subcutaneously into ICR mice. EAMP was injected intraperitoneally at 6 mg/kg (5 day/week × 2). The mice were sacrificed and then the tumors were dissected 28 days later after cell inoculation. Consequently, EAMP significantly inhibited the growth of sarcoma-180 *in vivo*: its inhibition ratio was 59.2% (Figure 2).

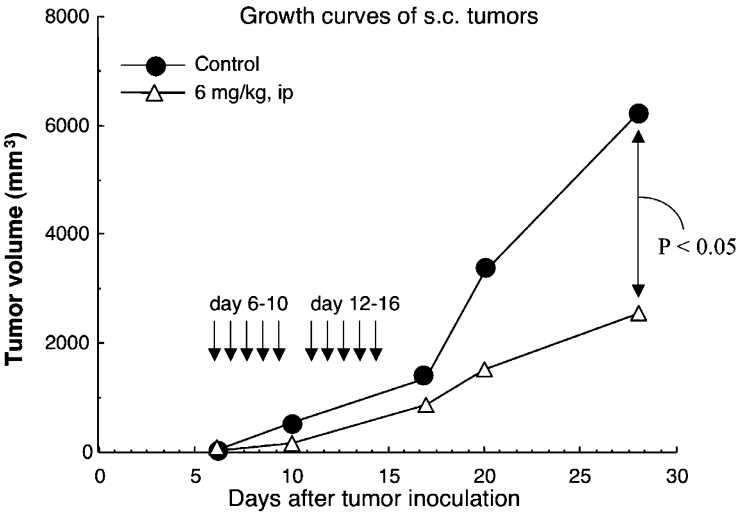

FIGURE 2 *In-vivo* antitumor activity of EAMP.

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CONCLUSION

EAdo may therefore be a potent lead compound for the development of a novel antitumor purine nucleoside analog which might be therapeutically effective for insensitive tumors against 2′-deoxycytidine and 2′-deoxyadenosine analogs that are preferentially phosphorylated by dCK. We propose that EAMP may be a useful prodrug of EAdo.

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