DEVELOPMENT OF AN ANTITUMOR ADENOSINE ANALOG, 3'-ETHYNYLADENOSINE

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Several 2'-deoxyadenosine analogs such as cladribine, fludarabine, and clofarabine are used in the treatment of lymphoid malignancies. 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). With the aim of developing more potent antitumor nucleosides than 2'-deoxyadenosine analogs reported, 3'-ethynyladenosine (EAdo) was designed as an RNA synthesis inhibitor.[1] We have studied the antitumor mechanisms of EAdo using human solid tumor cell lines. In culture, IC_{50} values of EAdo ranged from 0.05 μM to 2 μM. EAdo also effectively inhibited RNA biosynthesis in tumor cells. The cytotoxic activity of EAdo was markedly reduced by simultaneous treatment with the adenosine kinase (AK) inhibitor ABT-702. Furthermore, EAdo-resistant cells newly established from human gastric cancer cell line (NUGC-3) showed extremely decreased AK activity and expressed aberrant AK mRNA with deletion of 151 bp corresponding Exon 8 and Exon 9. These results indicate that cellular EAdo is metabolically phosphorylated by AK and that the AK gene becomes a molecular target for acquired resistance. On the other hand, we found that EAdo as well as adenosine and its analogs was enzymatically converted to inosine form (EIno) and rapidly inactivated by adenosine deaminase. As a result, the intraperitoneal administration of EAdo was able to prolong the survival time of mice bearing P388 leukemia, but was ineffective in mice bearing sarcoma-180 solid tumor. Thus, to improve the *in vivo* antitumor efficacy of EAdo we synthesized two EAdo derivatives (2-F-EAdo and EAdo-5'-monophosphate, EAMP). Although 2-F-EAdo was highly resistant to adenosine deaminase, its antitumor activity extremely depended on cellular AK activity because 2-F-EAdo showed the cytotoxic effect on only AK-overexpressing tumor cells. On the contrary, EAMP showed the growth inhibitory effect on sarcoma-180 solid tumor transplanted s.c. in mice. We propose that EAdo may be a potent lead compound to develop novel antitumor purine nucleoside analog which is therapeutically available for insensitive tumors to deoxycytidine and deoxyadenosine analogs preferentially phosphorylated by deoxycytidine kinase.

References

1. Endo Y, Obata T, Nomura M, Fukushima M, Yamada Y, Matsuda A, Sasaki T. (2007) Development of an antitumor adenosine analog, 3'-ethynyladenosine. *Nucleosides, Nucleotides and Nucleic Acids*. 26: 691-694.