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NOVEL DERIVATIVES OF 9-β-D-ARABINOFURANOSYLPURINES THAT ARE CYTOTOXIC TO VARIANTS OF HUMAN LEUKEMIA LYMPHOBLAST CELLS RESISTANT TO BOTH 1-β-D-ARABINOFURANOSYLCYTOSINE AND 9-β-D-ARABINOFURANOSYLADENINE

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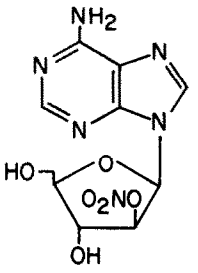
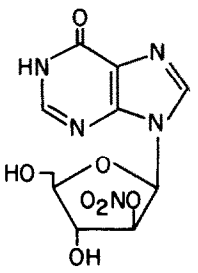
9-β-D-Arabinofuranosyladenine (ara-A) is a potent antiviral drug [1] that is being evaluated as an anticancer drug [2]. Its therapeutic effectiveness has been limited due to its rapid deamination by adenosine deaminase (ADA), present in body tissues and some neoplastic cells, to the less active 9-β-D-arabinofuranosylhypoxanthine (ara-H) [3]. Among experimental tumors, only those with relatively low ADA activity appear to be responsive to ara-A used as a single agent [4]. Furthermore, as with 1-β-D-arabinofuranosylcytosine (ara-C) [5,6], a drug commonly used in the treatment of acute leukemias [5], resistance to ara-A by cultured tumor cells stems partly from a deficiency of the required activating kinases [7,8]. Hence, deletion of the initial activating enzyme, deoxycytidine (dCyd) kinase, has been the most frequently identified biochemical basis for the resistance of cultured tumor cells to ara-C [5,6], while deletion of either dCyd or adenosine kinase or both can account for resistance of lymphoid cells to ara-A [7,8]. Attempts to minimize the problem of inactivation by enzymatic deamination of ara-A have included structural modifications of the drug [9-13], as well as the development of adenosine deaminase inhibitors to be used in combination with this agent [14,15]. These inhibitors, however, may produce multiple effects on cellular metabolism [16] and cause neurological and other toxicities in man [17]. Furthermore, inhibition of adenosine deaminase, by decreasing the catabolism of endogenous deoxyadenosine, might lead to elevation of intracellular pools of deoxyadenosine triphosphate in the target tumor cells, hence reducing the potential toxic effect of the triphosphate of ara-A [18,19]. Development of intrinsically deaminase-resistant analogs of ara-A is therefore highly desirable.

We recently reported an approach to circumvent an analogous problem with ara-C [20]. By introducing a neutral O-nitro group at the 2'-position of ara-C, we produced a compound, 2'-O-nitro-1-β-D-arabinofuranosylcytosine (nitrara-C), that is resistant to enzymatic deamination and has antileukemic activity both *in vitro* and *in vivo* [20]. Presented in this report is a brief description of the preliminary biological studies of the newly synthesized

2'-O-nitro derivatives of ara-A and ara-H -- 2'-O-nitro-9-β-D-arabinofuranosyladenine (2'-O-nitro-ara-A, hereafter nitrara-A) and 2'-O-nitro-9-β-D-arabinofuranosylhypoxanthine (2'-O-nitro-ara-H, hereafter nitrara-H).



Nitrara-A



Nitrara-H

Nitrara-A and nitrara-H are, respectively, about 4 and at least 1000 times more soluble in water (at room temperature) than ara-A. Hence, these compounds have a solubility advantage over ara-A. This advantage may be of practical importance, since the utility of ara-A as a chemotherapeutic agent is limited in part by its low solubility in water (about 0.5 mg/ml at room temperature) [9,12].

Table 1 shows the relative initial velocity of deamination of nitrara-A and some known substrates by mammalian ADA. The relative rates determined for adenosine, 2'-deoxyadenosine and ara-A agree well with the values reported by Robins and Hawrelak [12], while the ratio of rates for ara-A and 2'-azido-2'-deoxy-β-D-arabinofuranosyladenine (arazide)agrees well with that published by Lee et al. [9]. Although it is still a substrate for ADA, nitrara-A was deaminated about 25 times slower than ara-A.

Table 1. Substrate specificity of ADA*

Compound†	Relative initial rate‡
Adenosine	100§
2-Deoxyadenosine	118
Ara-A	18
Arazide	2.7
Nitrara-A	0.7

*ADA (adenosine deaminase, EC 3.5.4.4; Sigma Chemical Co., Type I) purified from calf intestinal mucosa. The amount of 2 x 10^{-4} mg of enzyme protein in a final volume of 1.0 ml of phosphate buffer (pH 7.4, 0.05 M) was used per assay.

†The concentration of each substrate was 1 x 10^{-4} M.

‡Direct spectrophotometric assay was performed at 25° by published procedure [9,12].

§Deaminase activity is expressed as a proportion of the natural substrate, adenosine, which was arbitrarily assigned a value of 100.

Table 2 shows that both nitrara-A and its deaminated product, nitrara-H, exerted appreciable cytotoxicity on the growth of the human T-lymphoblasts (CCRF-CEM, hereafter CEM) and their arabinonucleoside-resistant variants, CEM/ara-C and CEM/ara-C/ara-A, respectively. The cytotoxicity of ara-A in CEM cells was potentiated about 14-fold with the addition of 2'-deoxycoformycin (dCF), an effect that can be attributed to dCF-mediated inhibition of ara-A deamination [14]. By contrast, inhibition of the deamination of nitrara-A with dCF enhanced its cytotoxicity only slightly (less than 2-fold). Of particular interest is that both nitrara-A and nitrara-H were cytotoxic to variants of CEM cell lines which, because of a deficiency in certain nucleoside kinases, are resistant either to ara-C [6] or to both ara-C and ara-A [7]. Nitrara-A, in the absence of dCF, as well as nitrara-H, inhibited the growth of these two resistant lines as effectively as they did the wild type. Against CEM/ara-C/ara-A, nitrara-A was slightly less inhibitory in the presence of dCF than in its absence. Moreover, ara-H was cytotoxic to the parent CEM line but not to the resistant variants. Lack of response of ara-C-resistant lines (CEM/ara-C and CEM/ara-C/ara-A) to ara-H may be explained by loss of dCyd kinase in these cells, since ara-H is known to be a substrate for this enzyme partially purified from calf thymus [21]. The lack of cross-resistance of these two nitrate esters to the kinase-deficient lines suggests a specific mechanism of action for these new compounds that is distinct from that of ara-A, ara-H, or other known arabinofuranosylpurines, namely, arazide or 9-β-D-arabinofuranosyl-2-fluoroadenine (F-ara-A).

Table 2. Cytotoxicity of arabinofuranosylpurines against CEM cells and their kinase-deficient variants

Compound	IC_{50}* (μM)		
	CEM†	CEM/ara-C‡	CEM/ara-C/ara-A§
Nitrara-A	18	22	16
Nitrara-A + dCF‖	12	27	29
Nitrara-H	30	39	30
Ara-A	7.4	250	170
Ara-A + dCF‖	0.6	3.7	150
Ara-H	6	210	120
F-Ara-A	2.3	740	ND¶
Arazide	1.9	2	240

*Concentration required for 50% inhibition of cell growth. Each experiment was repeated two or three times with results of a single typical experiment presented in the table.

†The culture conditions were essentially similar to those described in Ref. 6.

‡Mutant deficient in dCyd kinase and resistant to ara-C by about 2000-fold. For general procedure of the growth of cells, see Ref. 6.

§Mutant deficient in both dCyd and adenosine kinases and resistant to both ara-C and ara-A. Cell culture methods have been described in Ref. 7.

‖Incubated in combination with 3.6 μM dCF to prevent deamination.

¶Not determined.

We have shown previously that F-ara-A, with a substitution of the hydrogen with a fluorine atom at the 2-position of ara-A, renders the molecule more specific than ara-A in its requirement for activation by dCyd kinase [6], a finding also confirmed by others [11]. Arazide, with a substitution of the hydroxyl group with an azido group at the 2'-position of ara-A, changed the substrate specificity of the new molecule toward activation by adenosine kinase (Table 2). On the other hand, the 2'-nitrate ester of ara-A did not appear to require activation by either of the two kinases. Moreover, such a replacement of the 2'-hydroxyl group with a nitrate group decreased the susceptibility of the compound to deamination. Although less potent, its deaminated product, nitrara-H, was also cytotoxic to CEM cells and their kinase-deficient variants. This lack of cross-resistance suggests that dCyd or adenosine kinase is not a limiting factor in the activation of either of the new nitrate esters in these human leukemic cells. Their cytotoxicity may be mediated, in part, by a mechanism involving S-adenosylhomocysteine hydrolase and thus interfering with transmethylation reactions [22]. The antineoplastic potential of both nitrara-A and nitrara-H in vivo is currently being investigated. Similarly, the possible mechanism of action of these two compounds is being studied.

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