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CYTOTOXIC ACTIVITY OF THE ADENINE ARABINOSIDE ANALOGS, 2'-AZIDO- AND 2'-AMINO-2'-DEOXY-β-D-ARABINOFURANOSYLADENINE

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9-β-D-Arabinofuranosyladenine (araA) is a purine nucleoside with activity against DNA viruses [1] and experimental neoplasms [2,3]; the compound is also currently being clinically evaluated as a cancer chemotherapeutic agent in man [4]. However, the efficacy of araA as an antitumor agent is limited by its rapid deamination by adenosine deaminase to 9-β-D-arabinofuranosylhypoxanthine which has no anticancer activity [2,5]. Attempts to circumvent the problem of rapid inactivation have included the development of potent inhibitors of adenosine deaminase [6-9] to employ in combination with araA and the structural modification of araA in an effort to prevent or minimize deamination [10,11].

A recent report [12] has described an approach to an analogous problem with 1-β-D-arabinofuranosylcytosine; modification of this structure to 2'-azido- and 2'-amino-2'-deoxy-β-D-arabinofuranosylcytosine results in agents with potent antineoplastic activity and resistance to inactivation by deamination by cytidine deaminase. This finding has prompted this preliminary investigation of the cytotoxic activity and susceptibility to adenosine deaminase of the corresponding derivatives of araA, 2'-azido-2'-deoxy-β-D-arabinofuranosyladenine (arazide) and 2'-amino-2'-deoxy-β-D-arabinofuranosyladenine (aramine) which were synthesized in our laboratory (Sandoz Forschungsinstitut).

Arazide and aramine were compared with araA as substrates for adenosine deaminase (EC 3.5.4.4) of calf intestinal mucosa and of cultured L1210 leukemia cells; both of these agents were inferior to araA as substrates for adenosine deaminase, as measured by a spectral decrease at 265 nm (Table 1). Thus, the rate of deamination of araA was 4.7-fold greater than that of arazide by adenosine deaminase of calf intestinal mucosa, whereas araA was only 2.9 times better than arazide as a substrate for the enzyme from L1210 cells. Aramine was also less efficacious as a substrate for adenosine deaminase than araA, but somewhat superior to arazide. Fifty percent inhibition of the growth of L1210 leukemia cells, as monitored by cell number, was caused by 5 and 20 μM arazide and aramine, respectively, in 24 hr, whereas 6 μM araA was required for the same inhibitory effect (Fig. 1).

Table 1. Substrate specificities of adenosine deaminase from calf intestinal mucosa and L1210 leukemia

Substrate	Enzyme activity*	
	Calf intestinal mucosa†	L1210 leukemia‡
AraA	19.2	0.023
Arazide	4.1	0.008
Aramine	7.9	0.011

*Enzyme activities were measured by a decrease in the absorbance of substrates at 265 nm. Reaction mixtures consisted of 0.8 ml of 0.05 M sodium phosphate buffer (pH 7.4) containing 50 μM substrate and an appropriate amount of enzyme (0.1 and 100 μg protein of calf intestinal mucosa and L1210 leukemia, respectively). Activity is defined as μmoles of substrate deaminated/min/mg of protein at 37°.
†Adenosine deaminase Sigma Type I.
‡Exponentially growing L1210 cells were collected, washed with 0.9% NaCl, and disrupted by sonication. Following centrifugation at 37,000 g for 30 min, the supernatant solution was used as the source of adenosine deaminase.

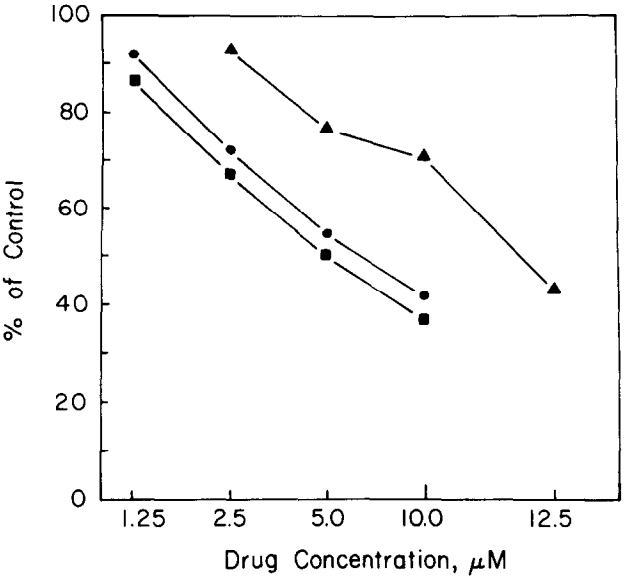

Fig. 1. Dose-response effects of araA, arazide and aramine on the proliferation of L1210 leukemia cells. Cells cultured in Fischer's medium containing 10% horse serum were incubated at 37° and their numbers determined with a ZBI Coulter particle counter 24 hr after exposure to drugs. Key: (●——●), araA; (■——■), arazide; and (▲——▲), aramine.

The antineoplastic activity of araA has been reported to be enhanced against Sarcoma 180, L1210 leukemia and Ehrlich carcinoma when used with inhibitors of adenosine deaminase [3,13-16]. In a similar manner, a noninhibitory concentration (1 μM) of aramine, araA and arazide caused 32, 47 and 81 percent inhibition, respectively, of the proliferation of L1210 cells measured at 72 hr when employed simultaneously with the adenosine deaminase inhibitor 2'-deoxycoformycin (2'-dCF, 0.2 μg/ml); these results are shown in Fig. 2. 2'-dCF

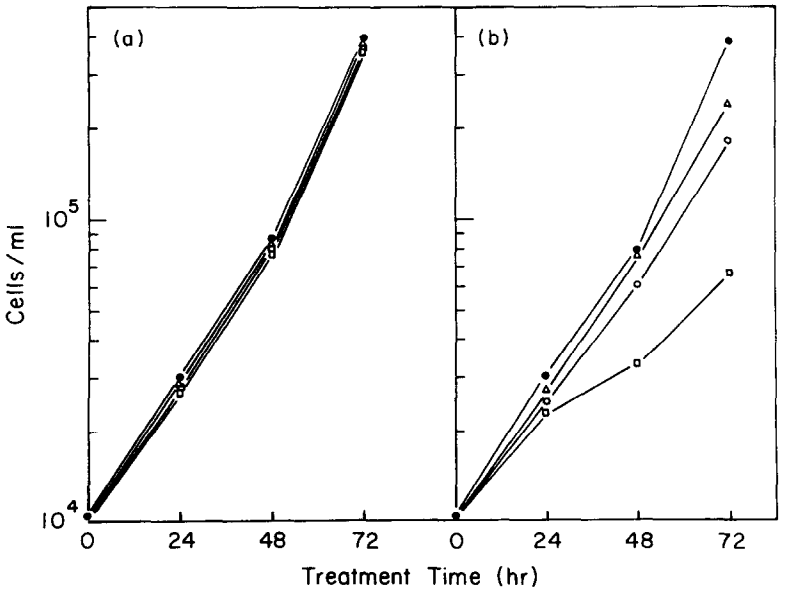

Fig. 2. Effects of araA, arazide and aramine in combination with 2'-dCF on the growth of L1210 cells in culture. Panel a: cells cultured in Fischer's medium containing 1.0 μM of each agent. Key: (●——●), untreated control; (○——○), araA; (□——□), arazide; and (Δ——Δ), aramine. Panel b: cells cultured in 1.0 μM of each agent in combination with 0.2 μg/ml of 2'-dCF. Key: (●——●), 2'-dCF; (○——○), 2'-dCF plus araA; (□——□), 2'-dCF plus arazide; and (Δ——Δ), 2'-dCF plus aramine.

increased the cytotoxicity of araA to L1210 cells in vitro, a finding that agrees with previous reports [14,15]. More marked potentiation of the activity of arazide and a lesser increase in the potency of aramine was produced by 2'-dCF. These findings suggest that the increase in the cytotoxic activity of both arazide and aramine caused by 2'-dCF is due to prevention of deamination of these compounds and that deaminated forms of these agents are either inactive or less active against these neoplastic cells. The differences observed in the degree of potentiation of the cytotoxic action of these nucleoside analogs by 2'-dCF suggest that arazide is considerably more potent than araA as an inhibitor of the growth of L1210 cells, while aramine is less active.

The relative rates of deamination of araA, arazide and aramine by adenosine deaminase from different sources are not equal (Table 1). Thus, the relatively small difference in cytotoxicity to L1210 cells of araA and arazide in the absence of 2'-dCF may be due in part to extensive deamination of arazide as well as of araA by adenosine deaminase from L1210 cells. It might be expected, however, that arazide will show greater cytotoxicity than araA in the absence of adenosine deaminase inhibitors in those tumor systems in which arazide has weak substrate activity for adenosine deaminase. A study of this possibility in various tumor systems, as well as studies to determine the therapeutic efficacy of arazide in tumor-bearing animals in the presence and/or absence of inhibitors of adenosine deaminase are currently being conducted; these latter investigations are particularly essential, since a variety of factors are involved in the potential usefulness of antineoplastic agents. Arazide has a solubility advantage over araA, being approximately 25-fold more water-soluble at room temperature; this advantage may be of importance, since the efficacy of araA as an antitumor agent is limited in part by its low water solubility [17].

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REFERENCES

1. R. E. Keeney and R. A. Buchanan, Ann. N.Y. Acad. Sci. 255, 185 (1975).
2. J. L. York and G. A. LePage, Can. J. Biochem. 44, 19 (1966).
3. R. Koshiura and G. A. LePage, Cancer Res. 28, 1014 (1968).
4. G. A. LePage, A. Khaliq and J. A. Gottlieb, Drug. Metab. Dispos. 1, 756 (1973).
5. J. J. Brink and G. A. LePage, Cancer Res. 24, 1042 (1964).
6. T. Sawa, Y. Fukugawa, I. Homma, T. Takeuchi and H. Umezawa, J. Antibiot., Tokyo (Ser. A) 20, 227 (1967).
7. P. W. K. Woo, H. W. Dion, S. M. Lange, L. F. Dahl and L. J. Durham, J. heterocyclic Chem. 11, 641 (1974).
8. H. J. Schaeffer and C. F. Schwender, J. med. Chem. 17, 6 (1974).
9. B. Evans and R. Wolfenden, J. Am. chem. Soc. 92, 4751 (1970).
10. J. A. Montgomery and K. Hewson, J. Am. chem. Soc. 79, 4559 (1957).
11. R. W. Brockman, F. M. Schabel, Jr. and J. A. Montgomery, Biochem. Pharmac. 26, 2193 (1977).
12. M. Bobek, Y. C. Cheng and A. Bloch, J. med. Chem. 21, 597 (1978).
13. G. A. LePage, L. S. Worth and A. P. Kimball, Cancer Res. 36, 1481 (1976).
14. C. E. Cass and T. H. Au-Yeung, Cancer Res. 36, 1486 (1976).
15. S. H. Lee, N. Caron and A. P. Kimball, Cancer Res. 37, 1953 (1977).
16. W. Plunkett and S. S. Cohen, Cancer Res. 35, 1547 (1975).
17. J. J. Brink and G. A. LePage, Can. J. Biochem. 43, (1965).