MOLECULAR PHARMACOLOGY, 14, 366-369

SHORT COMMUNICATION

Inhibition of Drug Metabolism by a Prodrug: 9-β-D-Arabinofuranosyladenine 5'-Valerate as an Inhibitor of Adenosine Deaminase

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(Received August 8, 1977)
(Accepted September 21, 1977)

SUMMARY

LIPPER, ROBERT A., MACHKOVECH, SUSAN M., DRACH, JOHN C. & HIGUCHI, WILLIAM I. (1978) Inhibition of drug metabolism by a prodrug: 9-β-D-arabinofuranosyladenine 5'-valerate as an inhibitor of adenosine deaminase. *Mol. Pharmacol.*, 14, 366-369.

9-β-D-Arabinofuranosyladenine 5'-valerate (ara-A-5'-valerate), a bioreversible derivative or prodrug of the antiviral agent 9-β-D-arabinofuranosyladenine (ara-A), was shown to be a competitive inhibitor of calf intestinal adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4). With ara-A as the substrate, apparent K_m and K_i values were 110 μM and 11 μM, respectively, at 37°. Ara-A-5'-valerate is not subject to adenosine deaminase-catalyzed deamination, and it is more water-soluble and more lipophilic than ara-A.

Bioreversible derivatives of pharmacologically active substances or "prodrugs" are frequently prepared as a means of overcoming physicochemical barriers to drug delivery (1-3). For example, prodrugs may be used to increase aqueous solubility (4) and/or lipophilicity (5). Prodrugs may also be used to prolong drug action by permitting slow regeneration of the active parent compound *in vivo* (6). Similar rationale has prompted the synthesis of drug derivatives (not necessarily reversible) that retain pharmacological activity and also avoid metabolic degradation which may befall the parent compound (7, 8).

Ara-A,[1] a nucleoside analogue, has demonstrated activity against a broad spectrum of DNA viruses (9-12), but its low aqueous solubility (0.4 mg/ml at 25°)[2] and low lipophilicity (log apparent pentanol/water partition coefficient = −0.48)[2] have limited its clinical usefulness (5, 13, 14). In addition, ara-A is rapidly metabolized by adenosine deaminase to ara-H, which has only about 1/30 the antiviral potency of ara-A (15-17).

The 5'-valerate ester of ara-A is more water-soluble (approximately 8 mg/ml at 25°)[2] and more lipophilic (log apparent pentanol/water partition coefficient = 1.33)[2] than ara-A and therefore was considered as a potential ara-A prodrug. In

This work was supported in part by Grant DE 00204-11 from the National Institute of Dental Research.

[1] The abbreviations used are: ara-A, 9-β-D-arabinofuranosyladenine [vidarabine, Vira-A (Warner-Lambert/Parke, Davis)]; ara-H, 9-β-D-arabinofuranosylhypoxanthine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

[2] D. C. Baker, personal communication.

0026-895X/78/0142-0366$02.00/0

addition, the presence of the ester moiety at the 5′-position should prevent the enzymatic deamination of the valerate (8), and this has in fact been shown to be the case.[2] In a separate study (18)[3] we have examined the transport and metabolism of ara-A-5′-valerate in suspension cultures of KB cells. Following cellular uptake, the valerate ester was not deaminated but was hydrolyzed to yield the parent drug, ara-A. Surprisingly, intracellular concentrations of ara-A were as high in cells treated with ara-A-5′-valerate as they were in cells treated with ara-A plus the adenosine deaminase inhibitor covidarabine (19). Thus, in cells treated with the valerate ester, it appeared that ara-A, once liberated, was not deaminated to any significant degree. In cells treated only with ara-A extensive deamination occurred. This observation led us to hypothesize that ara-A-5′-valerate itself was acting as an inhibitor of adenosine deaminase. Studies *in vitro* testing this hypothesis have been conducted, and the results are reported here.

The deamination of 22.2, 89.9, 226, and 459 μM ara-A (Pfanstiehl) by 0.05 unit/ml of calf intestinal adenosine deaminase (Boehringer/Mannheim) was monitored in pH 7.4 HEPES-buffered saline solution (20) at 37°. Each ara-A solution contained 100 μl of 56.1 μM [2-^{3}H]ara-A (New England Nuclear; specific activity, 17.7 Ci/mmole), resulting in specific activities of 332, 84.2, 33.8, and 9.68 mCi/mmole, respectively. At each ara-A concentration, the reaction was carried out in the presence of 0.00, 9.21, 46.1, 230, and 921 μM ara-A-5′-valerate (Warner-Lambert/Parke-Davis).

The reaction was initiated by addition of the enzyme and terminated by the addition of 500 μl of ice-cold USP ethanol. For each combination of ara-A and ara-A-5′-valerate concentrations, the reactions were conducted for 10 sec (taken as zero time) and 5 and 10 min. Under the reaction conditions used, ara-A-5′-valerate underwent no appreciable chemical decomposition throughout the duration of the experiment. The reaction mixtures were stored at −23° until they were assayed as described (18). Briefly, ara-A and ara-H were separated by thin-layer chromatography on silica gel GF plates (Analtech). The plates were developed by ascending chromatography at ambient temperature in chloroform–methanol–3% (v/v) aqueous acetic acid, 3:2:1, lower phase (21). Species of interest were localized by concurrent application of nonradiolabeled reference standards and visualization under 254 nm ultraviolet light following development. R_F values were: ara-H, 0.34; ara-A, 0.40. Appropriate portions of the adsorbent were scraped into liquid scintillation vials and eluted for 12 hr with 2 ml of 0.1 N hydrochloric acid in methanol. Aquasol (10 ml; New England Nuclear) was added to each vial and the radioactivity in the samples was determined by liquid scintillation counting (Beckman LS-200).

The data showing concentration of ara-A vs time were analyzed in order to permit computation of initial deamination rates. Cartesian and semilogarithmic linear regressions were performed on each data set, and the slope of the regression yielding the higher correlation coefficient was used to calculate the initial rate of disappearance of ara-A. The results indicate a marked decrease in the rate of ara-A deamination brought about by increasing ara-A-5′-valerate concentration. In the case of 22.2 μM ara-A, for example, the data reflect a nearly 80-fold reduction in initial deamination rate as the ara-A-5′-valerate concentration was increased from zero to 921 μM.

To elucidate the mechanism by which the deamination is inhibited by ara-A-5′-valerate, Lineweaver-Burk analyses were performed on the initial rate data. Double-reciprocal plots are shown in Fig. 1. The common positive intercept on the ordinate is indicative of competitive enzyme inhibition. Other standard plots, such as those of Hofstee or Woolf (22), also clearly suggest this mechanism. The Michaelis-Menten parameters describing the system were determined from the slopes and intercepts of the double-reciprocal plots.

[3] R. A. Lipper, S. M. Machkovech, W. I. Higuchi, J. C. Drach, and C. Shipman, Jr., manuscript in preparation.

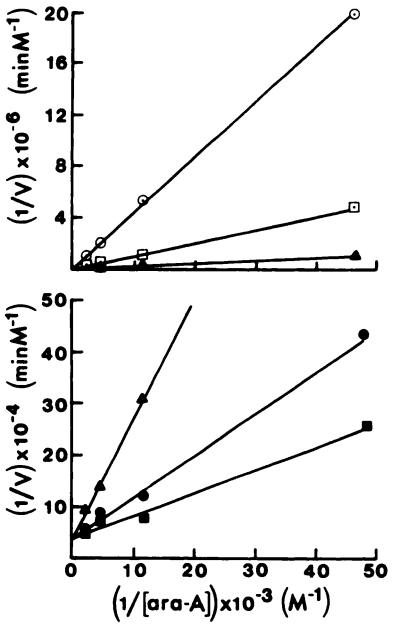

FIG. 1. *Lineweaver-Burk analysis of deamination of ara-A alone and in the presence of various ara-A-5'-valerate concentrations*

Initial rates of disappearance of 22.2, 89.9, 226, and 459 μM ara-A in the presence of 0.05 unit/ml of adenosine deaminase and 0 (■), 9.21 (●), 46.1 (▲), 230 (⊡), or 921 (⊙) μM ara-A-5'-valerate were determined by regression analyses of profiles of ara-A concentration vs. time (0–10 min). The reactions were carried out at 37°.

V_{max}, K_m, and K_i are 25 μM min^{-1}, 110 μM, and 11 μM, respectively.

Since different adenosine deaminases have been noted in different tissues and organisms (23, 24), the results *in vitro* do not directly prove the hypothesized enzyme inhibition by the valerate ester in the KB cell system. The data presented here, however, strongly support that hypothesis.

The demonstrated enzymatic inhibitory activity of ara-A-5'-valerate *in vitro* points toward a novel potential advantage of prodrugs, namely, that the liberated parent compound may be protected from metabolic degradation by the presence of the prodrug. With respect to compounds that are rapidly metabolized to less potent or inactive species, this phenomenon might prove clinically useful. The need for coadministration of a separate chemical entity or entities as enzyme inhibitor(s) (25, 26) might be eliminated. In addition, inhibition of metabolic enzymes by a prodrug is self-limiting; it serves to prolong the activity of the parent compound but eventually vanishes; the prodrug levels diminish, with the metabolism of the parent compound accelerating, until the system is cleared. In the particular case of ara-A, potential untoward effects of more potent (tightly binding) adenosine deaminase inhibitors should be avoided (27, 28).

ACKNOWLEDGMENTS

We thank Drs. D. C. Baker, A. J. Glazko, and T. H. Haskell of Warner-Lambert/Parke-Davis Pharmaceutical Research Division for their generosity in supplying compounds used in these studies.

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