Increased Cytotoxicity of N^6-(Δ^2-Isopentenyl)adenosine in Combination with Pentostatin Against L-1210 Leukemia Cells

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Abstract □ Pentostatin (I), a tight-binding inhibitor of adenosine deaminase, was evaluated in combination with the partially effective antitumor nucleoside N^6-(Δ^2-isopentenyl)adenosine (II) for cytotoxic activity against cultured L-1210 lymphocytic mouse leukemia cells. Although I alone (≤ 10 μg/ml) was ineffective, it significantly potentiated and prolonged the cytotoxic and cytostatic activities of II. The combination of I (2–10 μg/ml) with II (25 μg/ml) resulted in inhibition of cellular proliferation (80–96%) within 24 hr with maintenance at that level for an extended period of time due to the continued ability of I to prevent the facile deamination of the allylic side chain of II. This type of adjuvant chemoprotection has potential use for other labile oncologic agents.

Keyphrases □ Pentostatin—potentiation of N^6-(Δ^2-isopentenyl)-adenosine, cytotoxicity, mouse L-1210 leukemia cells □ N^6-(Δ^2-isopentenyl)adenosine—potentiation by pentostatin, cytotoxicity, mouse L-1210 leukemia cells □ Cytotoxicity—N^6-(Δ^2-isopentenyl)adenosine, potentiation by pentostatin, mouse L-1210 leukemia cells

Since its initial characterization by Woo *et al.* (1), pentostatin (2′-deoxycoformycin) (I), the tight-binding inhibitor of adenosine deaminase, has been shown to possess both antilymphocytic (2) and immunosuppressive activity (3) in several animal test systems. The ability of I to oppose or block the enzymatic deamination of several adenosine analogues to inactive forms (4) prompted clinical interest and utilization of the drug in the treatment of human chronic and acute lymphocytic leukemia. Recent studies have shown that I is of particular use in the treatment of acute lymphoblastic leukemia (5), as well as for chronic T-cell lymphocytic leukemia (6). Of special interest has been combination of I with the antiviral and antitumor agent vidarabine (7).

Other studies have revealed the existence of a correlation between the cytotoxicity of I and the accumulation of deoxyadenosine-5′-triphosphate in acute lymphocytic leukemia (8, 9), as well as the ratio of deoxyadenosine-5′-triphosphate to adenosine-5′-triphosphate in red blood cells. Another adenosine nucleoside analogue N^6-(Δ^2-isopentenyl)adenosine (II), used in the present investigation, has also been shown previously to possess growth-inhibitory effects on sarcoma-180 cells as well as some cytostatic action in treating human myeloblastic leukemia (10). Its immunosuppressive activity (11) has been linked to its ability to block the transport of preformed nucleosides at the level of transmembrane translocation in several mammalian species (11, 12). Moreover, it has been possible to prepare a serologically specific antibody to II (13).

Despite their potential for treating human leukemias, use of II and other adenosine antimetabolites has been limited due to their susceptibility to enzymatic degradation and subsequent inactivation by an enzyme system related to adenosine deaminase [N^6-(Δ^2-isopentenyl)-adenosine-aminohydrolase (14)]. This difficulty has been overcome, in great measure, by release of II at a controllable and predictable rate from a silicone rubber monolithic polymer matrix (15–17). This represented a successful attempt to match the rate of release of II against the aminohydrolase activity in tumor cells. The present study using I was begun to establish a means of preventing the degradation of II, thereby potentiating and prolonging its effectiveness against L-1210 leukemia cells.

EXPERIMENTAL

Drug Agents—The preparation of N^6-(Δ^2-isopentenyl)adenosine (II) was conducted as described elsewhere (11, 12, 16). Pentostatin [(*R*)-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydroimidazo-[4,5-*d*][1,3]diazepin-8-ol; I] was supplied[1]. Both agents were stored at −20° over silica gel.

Culturing of L-1210 Cells and Determination of Cell Number—Mouse lymphocytic leukemia cells (L-1210) were routinely cultured under sterile conditions in a 5% carbon dioxide atmosphere at 37°, with transfer during the mid-log phase of growth into 5 ml of fresh medium[2] (RPMI 1640 supplemented with L-glutamine plus 10% fetal calf serum) as described elsewhere (16), using wide-mouth culture flasks[3] to accommodate silicone polymeric membranes when used. Total cell number and viability values were determined using Turk's solution and trypan blue exclusion, respectively (16). Aliquots of each cell suspension, to which II and/or I was added at time zero, were aseptically removed for the determination of cell number and for centrifugation–filtration[4] to yield cell-free filtrates for high-performance liquid chromatographic (HPLC) determinations.

Analysis of I and II Content in Cell Growth Medium—High performance liquid chromatography using two reverse-phase columns[5] eluted with varying methanolic solutions, as described in part previously (15, 16), was used to determine the stability and metabolism of both nucleoside drug agents in the cell culture medium at various intervals. This was accomplished during continuous incubation conditions by removal of aliquots (0.05–0.20 ml) for centrifugation–filtration (16).

Determination of the Cytotoxicity of I and II—Solutions of I or II were freshly prepared by dissolving the drugs in RPMI 1640 without serum, with sterile-filtration followed by aseptic addition at time zero.[6] At appropriate times thereafter, aliquots of each cell suspension were removed for determination of cell number, viability, and/or required analytical studies (16).

RESULTS AND DISCUSSION

Adenosine deaminase and a related aminohydrolase for II not only have important roles in normal catabolic pathways for adenine nucleosides, but are capable of deaminating N^6-(Δ^2-isopentenyl)adenosine (II) (14). Deamination of II to inosine greatly reduces its ability to interfere with nucleic precursor transport and synthesis (11, 12) and its immunosuppressive activity (11, 13), thereby diminishing its chemotherapeutic potential as an anticancer agent (10). The present investigation demonstrates that a natural product, the potent adenosine deaminase inhibitor pentostatin (1–9), can potentiate and prolong the antileukemic effect of

[1] Natural Products Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.
[2] Grand Island Biological Co., Grand Island, N.Y.
[3] Corning no. 25100; 25 ml.
[4] Bioanalytical System, Inc., West Lafayette, Ind.
[5] Micropak MCH-10, Varian Instruments Co., Palo Alto, Calif.; Zorbax ODS, Dupont Co., Wilmington, Del.
[6] Millex SLG-S0250S, Millipore Filter Co., Bedford, Mass.

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Table I—Effects of Pentostatin and N^6-(Δ^2-Isopentenyl)adenosine on the Proliferation of L-1210 Leukemia Cells in Culture [a]

	Compound Added [b]						
	None	I		II		I plus II	
Time, hr [c]	Cells/ml × 10^{-5}	Cells/ml × 10^{-5}	Inhibition, %	Cells/ml × 10^{-5}	Inhibition, %	Cells/ml × 10^{-5}	Inhibition, %
24	3 ± 0.5 [d]	3 ± 0.5	0	0.8 ± 0	73	0.5 ± 0	83
48	14.5 ± 1.0	14 ± 0.8	3.2	4.5 ± 0.5	69	2.8 ± 0.5	81
72	34 ± 1.0	33.5 ± 1.0	1.5	11.1 ± 1.0	68	4.5 ± 0.5	86
96	42 ± 1.5	41 ± 2.0	2.4	14.2 ± 1.0	66	9 ± 1.0	79
144	50 ± 1.5	48 ± 2.0	4.0	22 ± 1.0	56	7.5 ± 1.0	85

[a] Each T-flask initially contained 2 × 10^5 L-1210 mouse leukemia cells per milliliter (100% viability by trypan blue exclusion) in 5 ml of growth medium (RPMI 1640 plus 10% fetal calf serum). Experiments were conducted using 2 or 3 replicates for each type of determination. [b] Additions (0.1–0.3 ml) of each agent dissolved in RPMI 1640 medium followed by sterile filtration were made at time zero to yield final concentrations of I (2 μg/ml) and/or II (25 μg/ml). [c] Aliquots (0.1–0.2 ml) of each cell suspension were removed aseptically at various time intervals for the following determinations: total cell count using Turk's solution, cell viability (trypan blue exclusion), and HPLC (15, 16). [d] Mean ± *SD*.

II against cultured L-1210 cells (Table I). Although I alone (≤10 μg/ml) does not interfere with L-1210 cellular proliferation, it is capable of enhancing the antileukemic effects of II (25 μg/ml). At an optimal concentration of 5–10 μg/ml, I in combination with II results in almost total cell death (96%) within 24 hr. The few remaining cells have viability values of 40–50%. The most impressive effect of I (2 μg/ml) is its ability to prevent the cytotoxic capacity of II from declining at longer intervals of incubation time, when L-1210 leukemia cells are in the stationary phase of growth. This effect is discernible at concentrations of as low as 0.2 μg/ml[7]. These results suggest that inhibition of N^6-(Δ^2-isopentenyl)-adenosine-aminohydrolase prevents the inactivation of II, thereby enhancing and prolonging its effectiveness as an antitumor drug agent. In other recent studies (15–17), it has been demonstrated that the usefulness of II against L-1210 cells may also be potentiated by controlled release of this nucleoside from a polymeric silicone matrix.

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Arylidenepyruvic Acid Thiosemicarbazone and Thiazoline Derivatives As Potential Antimicrobial Agents

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Abstract □ Two novel series of arylidenepyruvic acid thiosemicarbazone and thiazoline derivatives were synthesized and evaluated as potential antimicrobial agents. These substances did not exhibit any significant antibacterial effects when tested against a variety of microorganisms.

Keyphrases □ Antimicrobial agents, potential—arylidenepyruvic acid thiosemicarbazone and thiazoline derivatives, synthesis, evaluation for antibacterial activity □ Arylidenepyruvic acids—thiosemicarbazones and thiazolines derivatives, synthesis, antimicrobial effects.

The introduction of a thiosemicarbazone moiety to alter the pharmacological activity of a variety of biologically active compounds has been demonstrated recently in several studies from this laboratory (1–5). Continuing such studies, the thiosemicarbazones (II–XIV) derived from various arylidenepyruvic acids and the corresponding thiazolines (XV–XXVII, Scheme I) were synthesized and tested for antimicrobial activity.

RESULTS AND DISCUSSION

Chemistry—The thio compounds (II–XXVII) were prepared as shown in Scheme I. A mixture of pyruvic acid or the properly substituted arylidenepyruvic acid (I), prepared through Claisen condensation of pyruvic acid and various aryl aldehydes (6), and an equivalent amount of 4-substituted 3-thiosemicarbazide was heated under reflux in aqueous acetic acid. The products (II–XIV) which separated on concentrating and cooling the mixtures, were crystallized from ethanol. The reaction of these

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