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Biochemical and Pharmacological Effects of the Fermentation-derived Antitumor Agent, (αS,5S)-α-Amino-3-chloro-4,5-dihydro-5-isoxazoleacetic Acid (AT-125)[1]

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ABSTRACT

(αS,5S)-α-Amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid (AT-125; α-amino-3-chloro-2-isoxazoline-5-acetic acid; U-42126; NSC 163501) is a fermentation-derived amino acid antimetabolite with significant antitumor activity. It has been shown previously to act in mammalian cells as a glutamine antagonist. In the present studies, AT-125 has been shown to exhibit sex-related toxicity towards mice; females are considerably more sensitive than males. Further, especially in male mice, toxicity appears to be age related, younger animals being more sensitive. Similar effects have been observed previously with another antitumor agent, 3-deazauridine, suggesting common biochemical mechanisms of action. Coadministration of testosterone with AT-125 alleviates the toxicity of the agent and, in L1210 leukemic mice, allows administration of higher doses and a resulting enhanced therapeutic activity. Cytidine triphosphate synthetase, a glutamine-dependent amidotransferase and the primary locus of 3-deazauridine activity, was also shown to be inhibited strongly by AT-125 (K_i 2 × 10^{-6} M). The effects of AT-125 on L1210 cell ribonucleotide pools (elevation of uridine triphosphate levels, decreases in cytidine triphosphate and guanosine triphosphate levels) were consistent with such inhibition and also suggested that inhibition of another glutamine-dependent enzyme, xanthosine monophosphate aminase, might be important in the action of this agent. Further support for such a hypothesis was provided when it was observed that a combination of cytosine and guanosine ribonucleosides (or deoxyribonucleosides) acted synergistically in reversing the growth-inhibitory activity of AT-125 towards L1210 cells in culture.

INTRODUCTION

AT-125[3] (Chart 1) was isolated from fermentation broths of *Streptomyces sviceus* (4, 8). It was found to have significant activity against L1210 leukemia in mice, providing increases in life span in excess of 100%, depending upon the schedule of administration (5, 10). AT-125 has also shown activity against P388 mouse leukemia and against human breast and lung tumor xenografts in athymic "nude" mice (6). The inhibition of L1210 cell growth in culture by AT-125 is antagonized by L-glutamine, and the agent has been shown to inhibit various bacterial and mammalian enzymes which catalyze the transfer of the amide group of L-glutamine (7).

Because of its promising antitumor activity, we have continued studies on the biochemical pharmacology of AT-125. In this communication, we report its greater toxicity to female as compared to male mice, the alleviation of this toxicity by testosterone, the kinetics of inhibition of CTP synthetase (EC 6.3.4.2) by this agent, and its effect on ribonucleotide pools in L1210 cells. The coincidence of these effects with those produced by the antitumor agent 3-deaza-Urd (1, 2, 9, 13) suggests a similarity in the mode of action of the 2 compounds.

MATERIALS AND METHODS

Materials. AT-125, testosterone, and testosterone 17β-cypionate were provided by The Upjohn Company, Kalamazoo, Mich. 3-Deaza-Urd was obtained from Dr. R. K. Robins, ICN, Irvine, Calif.

Chemotherapy Studies. The animals used for these studies were C57BL/6 × DBA/2 F_1 (hereafter called B6D2F_1) mice. Groups of 8 to 10 mice were housed in plastic cages and given pelleted feed and water *ad libitum*. L1210 leukemia was maintained by continuous i.p. passage in syngeneic female DBA/2 mice. Standardized protocols of the Drug Research and Development Program, National Cancer Institute, were followed for continuous passage of the tumor and for implantations of tumors into B6D2F_1 mice for chemotherapy experiments (3). Leukemic cells (1 × 10^5/mouse) were inoculated i.p. on Day 0, and treatment was initiated 24 hr later. AT-125 was administered i.p. in 0.2 or 0.5 ml of 0.9% aqueous NaCl. Treatment was daily for 9 consecutive days. Where the combination of testosterone and AT-125 was used, testosterone was administered s.c. in 0.2 ml cottonseed oil less than 5 min before AT-125. AT-125 doses were adjusted for the average weight of the experimental group on the first day of administration. In collateral sensitivity studies (Table 4), L1210 cells resistant to ara-C were also used. In these studies, the cell inoculum was 1 × 10^6 cells/mouse i.p. The percentage increases in life span were calculated from mean survivals. Animals were weighed (as groups) on Days 1 and 5, and average weight

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[3] The abbreviations used are: AT-125, (αS,5S)-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid (α-amino-3-chloro-2-isoxazoline-5-acetic acid; U-42126; NSC 163501); deaza-Urd, 3-deazauridine; ara-C, 1-β-D-arabinofuranosylcytosine.

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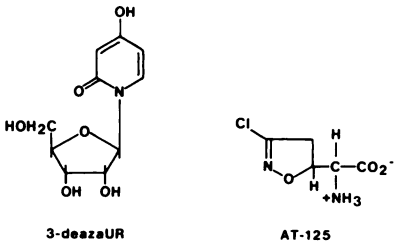

Chart 1. Structures of AT-125 and 3-deaza-Urd (*3-deazaUR*).

changes (g/mouse) were calculated.

Toxicity Studies. The toxic effects of AT-125 were determined in male and female ICR mice. The animals were grouped (6/group) by sex and weight, and the agent was administered i.p. at the doses (calculated from initial animal weights) and schedules shown in the tables. The mice were given free access to food and water. The 50% lethal doses were estimated by linear interpolation of a plot of percentage of survival at Day 14 against log dose. The dose of the agent which resulted in the death of no more than 1 of the 6 animals thus treated was considered the "maximally tolerated dose." Where testosterone 17β-cypionate was used, it was administered s.c. in cottonseed oil less than 5 min before AT-125.

***In Vitro* Inhibition Analysis.** The *in vitro* reversal studies were carried out by introducing 0.25-ml aliquots of Roswell Park Memorial Institute Medium 1640 containing AT-125 at 4 times the desired final concentration into 16- x 125-mm screw-cap culture tubes, followed by 0.25-ml aliquots of the medium containing the natural pyrimidine or purine nucleosides at 4 times their desired final concentration. Aliquots of the medium (0.5 ml) containing 3×10^5 L1210 leukemia cells were added to give a final volume of 1 ml. The cultures were incubated at 37° for 40 hr during which time the cell number in the controls increased approximately 8- to 9-fold, with a cell viability of 98 to 99% as determined by trypan blue exclusion.

Purification and Assay of CTP Synthetase. CTP synthetase from rat liver was purified approximately 500-fold by procedures described previously (9), except that the enzyme was dialyzed for 24 hr against 0.035 M Tris-HCl, pH 7.4, containing 10 mM L-glutamine and 0.1 M 2-mercaptoethanol to remove the ammonium sulfate used for fractionation. The enzyme activity was determined for 1 hr at 37° in a mixture of pH 7.2 containing (μmol/ml): ATP, 8 [2-^{14}C]UTP, 0.2 (1.0×10^6 dpm/pmol); GTP, 0.2; L-glutamine, 55; 2-mercaptoethanol, 50; $MgCl_2$, 18; Tris-HCl, 35; and the enzyme at the concentrations given in the appropriate charts. GTP serves as an allosteric activator of the enzyme; in its absence, the activity is reduced by 85% (12).

Intracellular Nucleotide Pools. AT-125 was added to 250 ml of a culture of L1210 cells (8×10^5 cells/ml of Roswell Park Memorial Institute Medium 1640) to provide a final concentration of 5×10^{-6} M. At the time points indicated, aliquots containing 2.5×10^7 cells were removed. The cells in each aliquot were collected by centrifugation at 4°, and 620 μl of 1 M perchloric acid were added to pellet. After stirring and extraction for 15 min, the suspension was centrifuged and reextracted with 225 μl of perchloric acid. To the combined extracts, 50 μl of 0.5 M Tris-HCl, pH 8.3, were added, followed by addition of 100 μl of 8 N KOH in 25-μl increments. The pH was adjusted to 8.0, salt was removed by centrifugation, and the supernatant was evaluated for ribonucleotide concentrations using a Dupont 830 high-pressure liquid chromatograph. The Permaphase ABX column was eluted with a linear gradient of 2 mM KH_2PO_4, pH 3.0, to a final concentration of 0.5 M KH_2PO_4, pH 6.0, at a rate of 4%/min.

RESULTS

During studies with AT-125 in experimental mouse tumors (in particular, L1210 leukemia), it became apparent that the therapeutic effects achieved were dependent on the sex of the mice used. The agent appeared to be more effective in male mice; the increase in their life span exceeded that of the females. Further, heavier (older) mice of either sex showed superior therapeutic responses. With male mice with an average weight of 23.5 g, the optimal therapeutic activity observed was higher (90% increased life span) than that observed in the same experiment with 17-g female mice (48% increased life span) (Chart 2). The optimal dose for the males (6.3 mg/kg/day) was 8 times that for the females (0.8 mg/kg/day) (Chart 2*a*). The precipitous decrease in the life span of female mice at higher doses of AT-125, which are therapeutically quite effective in the male animals, paired with the demonstration (Chart 2*b*) that such doses produce a much greater weight loss in the female as compared to the male animals, suggested that the toxicity of the agent is related to the sex of the tumor-bearing host. Studies in nonleukemic mice were carried out to further delineate these effects. As shown in Table 1, AT-125 is not

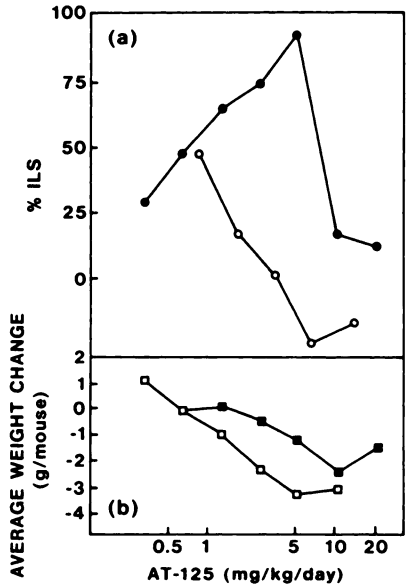

Chart 2. Therapeutic activity of AT-125 in male and female L1210-leukemic B6D2F$_1$ mice. The experiment was carried out with male mice, average weight (Day 1) of 23.5 g (●, ■), and female mice, average weight (Day 1) of 17 g (○, □). The same AT-125 solutions were used for male and female mice (0.5 ml i.p., daily for 9 days beginning Day 1 after tumor inoculation on Day 0), and doses were calculated based on Day 1 weights. Percentages of increase in life span (% *ILS*) were calculated from mean survival times of treated and control groups (*a*). Control mean survivals were 8.9 days (males) and 9.0 days (females). Mice were weighed on Days 1 and 5, and average weight changes were calculated (*b*).

Table 1
Sex- and weight-related toxicity of AT-125 in ICR mice

Sex	Av. wt[a] on Day 1 (g/mouse)	Dose range evaluated (mg/kg/day)	LD_{50}[b] (mg/kg/day for 9 days)	MTD (mg/kg/day for 9 days)	Daily AT-125 dose (mg/kg for 9 days) for: 20% wt loss[c] Days 1–6	Daily AT-125 dose (mg/kg for 9 days) for: No wt loss Days 1–6
Male	14.6	1.1–16.7	1.5	<1.1	2.1	1.6
	20.3	0.78–12.5	4.5	1.5	11	6.2
	24.3	0.65–10.5	~15	5.1	>10.5	7.5
Female	13.9	1.1–17.4	~0.8	<<1.1	1.7	~0.8
	19.3	0.78–12.8	1.3	0.78	2.4	1.0
	23.5	0.66–10.8	2.0	1.3	5.1	1.4

[a] Based on 6 groups of 6 mice each (see "Materials and Methods").
[b] LD_{50}, 50% lethal dose obtained by linear interpolation based on the number of Day 14 survivors; MTD, maximum dose at which >5 of 6 treated mice survived based on Day 14 survivors.
[c] Based on the average weight on Day 1.

only more toxic to females than to male mice but is also more toxic to small (young) animals than to heavier (older) ones. This is particularly apparent in the male mice, in which AT-125 is 5 to 10 times more toxic to young than to older animals; in female mice of comparable age, the difference in only 2- to 3-fold.

It had been observed previously (2) that another antileukemic agent, 3-deaza-Urd (Chart 1), is also more toxic to female than to male mice. Further, 3-deaza-Urd toxicity could be prevented by the administration of testosterone. Consequently, it was considered possible that the toxicity of AT-125 to the murine host might also be amenable to hormonal modification. As shown in Table 2, testosterone (administered as the 17β-cypionate) does indeed alleviate the host toxicity and lethality of AT-125 in both male and female ICR mice. This finding suggests that the greater toxicity of the drug to young (as compared to older) mice is a reflection of the hormonal status of these animals.

When evaluated in L1210-leukemic male $B6D2F_1$ mice (Chart 3), coadministration of testosterone appeared to allow the administration of higher doses of AT-125 (which would have been toxic if administered alone), resulting in an enhanced therapeutic effect. Similar effects of testosterone were also observed with female leukemic mice (data not shown).

As in the case of 3-deaza-Urd (3), the toxicity of AT-125 was directed primarily against the intestinal epithelium of the mouse, as evidenced by severe dehydration, diarrhea, shortened villi, and degradation of crypt cells. At the same time, some atrophy of the spleen with karyorrhexis of lymphoid elements was also seen.

Because of the accumulated evidence which suggests that deaza-Urd exerts its antitumor activity and host toxicity through the inhibition of CTP synthetase (9), paired with the close biological similarities of 3-deaza-Urd and AT-125, the effect of AT-125 on the activity of this enzyme was evaluated. In previous studies (7) with the use of crude rat liver homogenates and high concentrations of AT-125 (1 mM), inhibition of this enzyme had been observed. As shown by the Dixon plot in Chart 4, AT-125 interferes with the activity of an approximately 500-fold-purified CTP synthetase from rat liver with a K_i of 2×10^{-6} M. The plot is linear over the range of the inhibitor concentrations examined, indicating pure, not partial, inhibition. Using a second enzyme preparation, a K_i of 3×10^{-6} M was obtained. The K_i value calculated from the Lineweaver-Burk plot (Chart 5) is 7×10^{-7} M. Based upon all determinations, an average K_i of 2×10^{-6} M was obtained, a value which approximates the K_i of 5×10^{-6} M for 3-deaza-uridine triphosphate (9).

As demonstrated in Chart 5, AT-125 inhibition of CTP synthetase activity is competitive with L-glutamine, a cofactor in the enzymic reaction. Since glutamine is a cofactor for several other amidotransferases (7), a number of which have also been shown to be inhibited by AT-125, the proposition that AT-125 exerts its host toxicity and antitumor activity primarily by interfering with CTP synthetase activity requires experimental support. As one parameter for obtaining such support, the effect of AT-125 on the size

Table 2
Single-dose toxicity of AT-125 in ICR mice: effect of sex, weight (age), and testosterone administration

Mice surviving AT-125, 200 mg/kg (Day 14 survivors/total)							
Male mice				Female mice			
AT-125 alone[a]		AT-125 + testosterone[b]		AT-125 alone[a]		AT-125 + testosterone[b]	
Wt[c]	Survivors	Wt[c]	Survivors	Wt[c]	Survivors	Wt[c]	Survivors
13.9	1/8	14.0	7/8	13.7	4/8	12.7	0/8
16.1	4/10	16.3	6/10	15.6	1/10	15.5	4/10
18.1	2/8	19.0	8/8	18.3	2/8	18.5	7/8
18.7	10/10	18.4	10/10	17.1	0/10	17.4	10/10
21.8	10/10	21.5	9/10	20.6	10/10	19.8	8/10
22.0	8/8	22.0	8/8	23.2	2/8	22.4	8/8
24.8	10/10	25.0	10/10	25.1	3/10	24.4	8/10
25.9	10/10	26.8	10/10	26.0	4/10	26.3	10/10
Total	55/74		68/74		26/74		55/74
	(74%)		(92%)		(35%)		(74%)

[a] AT-125, 200 mg/kg i.p. in 0.5 ml 0.9% NaCl solution.
[b] AT-125, 200 mg/kg i.p. in 0.9% NaCl solution administered immediately after s.c. administration of testosterone 17β-cypionate, 50 mg/kg in sterile cottonseed oil.
[c] Average weight of group of treated animals on day of treatment.

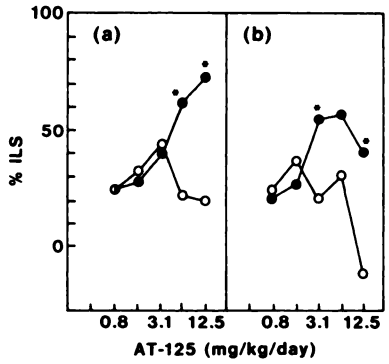

Chart 3. Effect of testosterone on AT-125 activity in L1210-leukemic mice. Male B6D2F$_1$ mice with average weights of 20.1 g (Experiment 1; 8/group) or 18.7 g (Experiment 2; 10/group) were treated daily i.p. with AT-125 for 9 days beginning 24 hr after i.p. inoculation of 10^6 L1210 cells/mouse (○). In addition, other groups (●) also received concomitant treatment with testosterone (50 mg/kg/day s.c. in cottonseed oil). Percentages of increase in life span (% *ILS*) were calculated from mean survival times of treated groups and control groups. Control animals for AT-125 treatment alone (○) received 0.9% NaCl solution and survived for 9.3 days (Experiment 1) and 8.8 days (Experiment 2). Controls for groups treated with both AT-125 and testosterone (●) received testosterone. Their survivals were 9.6 days (Experiment 1) and 8.4 days (Experiment 2). *, statistically significant differences ($p < 0.05$, Student t test) between groups receiving both agents and those receiving AT-125 alone.

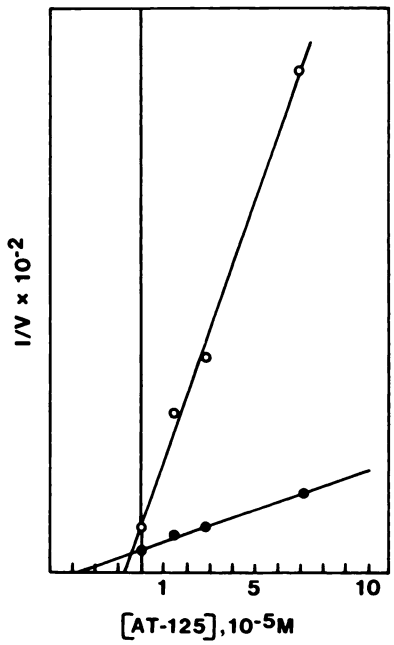

Chart 4. Dixon plot of rat liver CTP synthetase activity as a function of AT-125 concentration. ●, 7 mM L-glutamine; ○, 1 mM L-glutamine.

of the ribonucleotide pools of L1210 cells was examined. As shown in Table 3, following a 4-hr exposure of L1210 cells in culture to 5 μM AT-125, UTP concentrations were increased by 28% while CTP concentrations fell by 32%. During the same time, GTP pools decreased by 47% while ATP and ITP pools remained virtually unchanged.

In order to assess the relative contribution of the observed decrease in the CTP and GTP pools (and possibly their deoxyribonucleotide counterparts) to the inhibition of cell growth, cytidine, deoxycytidine, guanosine, and deoxyguanosine at 2.5×10^{-5} M were added alone or in combination to cultures of leukemia L1210 cells containing 2×10^{-5} M AT-125. As measured after 24 hr incubation, cytidine or deoxycytidine reversed the inhibition of growth by only 10 to 15%, whereas guanosine or deoxyguanosine effected a 27 to 30% reversal. When used in combination, the inhibition was decreased by 54 to 60%.

Because resistance to ara-C in L1210 cells was found to be associated with pronounced collateral sensitivity to 3-deaza-Urd, it was of interest to ascertain whether such an effect extends to AT-125 as well. As shown by the data in Table 4, this is not the case; the ara-C-resistant cells were only slightly more sensitive to the inhibitory effect of AT-125 than were the nonresistant cells.

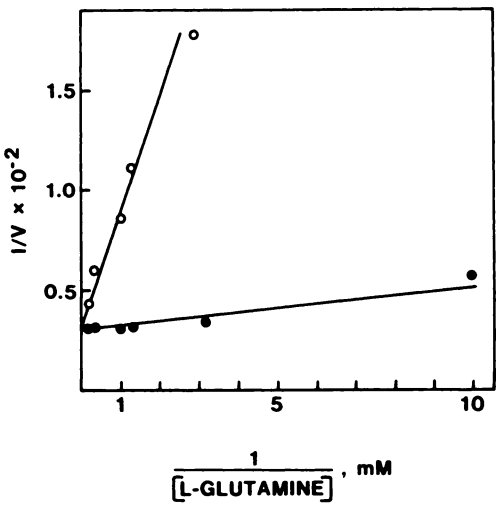

Chart 5. Effect of AT-125 on rat liver CTP synthetase activity as a function of L-glutamine concentration. ● control; ○, 1.4×10^{-5} M AT-125.

Table 3

Nucleotide pools in leukemia L1210 cells treated with 5 μM AT-125

	pmol/10^6 cells				
	GTP	ATP	ITP	UTP	CTP
2 hr					
Control	737	2208	271	1118	937
Drug-treated	525	2072	360	1806	781
4 hr					
Control	737	1799	266	1083	906
Drug-treated	395	1901	308	2471	620

Table 4

Comparison of effects of AT-125 and 3-deaza-Urd on L1210 leukemia sensitive to and resistant to ara-C

Male B2D6F$_1$ mice were treated daily (i.p.) for 9 days beginning 1 day after inoculation (10^6 cells) with L1210 cells or a variant, L1210/ara-C^r, resistant to ara-C. Control mean survivals were 6.8 days (L1210) and 7.3 days (L1210/ara-C^r), respectively.

Agent	Daily dose (mg/kg)	% increased life span	
		L1210	L1210/ara-C^r
ara-C	20	131	7
3-deaza-Urd	50	59	(3/6 cured)
	25	43	(3/6 cured)
AT-125	6.25	69	101
	3.13	81	108

DISCUSSION

The fact that 2 compounds (AT-125 and 3-deaza-Urd) of quite dissimilar structure possess similar antitumor properties and give rise to sex-related host toxicity modifiable by testosterone served as the stimulus for evaluating the biochemical-pharmacological basis of these effects. The primary activity of 3-deaza-Urd (after its metabolic conversion to 3-deaza-UTP) appears to be the inhibition of CTP synthetase (9). The fact that AT-125 (at the cofactor level) is an equally potent inhibitor of the enzyme (Charts 4 and 5) supports the suggestion that the similar effects of the 2 compounds may derive from the inhibition of this common metabolic site. Although AT-125 has been shown to inhibit a number of additional enzymes along the pyrimidine and purine *de novo* paths (7), including ATP:carbamate phosphotransferase, L-aspartate:L-glutamine amidoligase, and ribosylamino-5-phosphate:pyrophosphate phosphoribosyltransferase, these would appear to be ruled out as primary targets, since both the total pyrimidine and the total purine triphosphate pools are maintained in the presence of the inhibitor, providing evidence of continued *de novo* synthesis.

The reversal data obtained in leukemia L1210 cells *in vitro* would appear to suggest that, in contrast to 3-deaza-Urd which inhibits only CTP synthetase, AT-125 interferes with both CTP synthetase and XMP aminase activity (7). The fact that the inhibitory effect of the agents exerted against L1210 cells is apparently not prevented by testosterone, whereas the intestinal toxicity that they cause is readily alleviated by the hormone, might indicate not only that the intestinal epithelium is a target for testosterone but also that the predominant effect of the 2 agents in this tissue may be mediated via the inhibition of CTP synthetase activity. This interpretation proceeds, of course, on the assumption that nonspecific mechanisms, such as decreased uptake of the 2 drugs in the presence of the hormone, can be excluded.

The lack of collateral sensitivity of an ara-C-resistant L1210 cell line to AT-125 can be explained readily. The collateral sensitivity to 3-deaza-Urd has been shown to be associated with increased phosphorylation of this agent in the ara-C-resistant line (11), and because AT-125 does not require such metabolic activation an enhancement of its activity would not be expected under these circumstances.

In conclusion, whereas the similar biological effects of the 2 agents in the systems evaluated may be mediated via the same mechanism, the fact that AT-125, unlike 3-deaza-Urd, can inhibit a variety of glutamine-dependent amidotransferases suggests that its biochemical and biological action might vary depending upon the relative concentration and metabolic importance of an amidotransferase in a given target tissue. Additionally, differences in the pharmacokinetic behavior of the 2 agents might be expected; therefore, the therapeutic potential of both agents merits clinical evaluation.

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REFERENCES

1. Bloch, A., Dutschman, G., Currie, B. L., Robins, R. K., and Robins, M. J. Preparation and biological activity of various 3-deazapyrimidines and related nucleosides. J. Med. Chem., *16:* 294–297, 1973.
2. Bloch, A., Dutschman, G., Grindey, G., and Simpson, C. L. Prevention by testosterone, of the intestinal toxicity caused by the antitumor agent 3-deazauridine. Cancer Res., *34:* 1299–1303, 1974.
3. Geran, R., Greenberg, N. H., McDonald, M. M., Schumacher, A. M. and Abbott, B. J. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. Cancer Chemother. Rep., Part 3, *3:* 1–103, 1972.
4. Hanka, L. J., and Dietz, A. U-42,126, a new antimetabolite antibiotic: production, biological activity, and taxonomy of the producing microorganism. Antimicrob. Agents Chemother., *3:* 425–431, 1973.
5. Hanka, L. J., Martin, D. G., and Neil, G. L. A new antitumor antimetabolite, (αS,5*S*)-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid (NSC-163501): antimicrobial reversal studies and preliminary evaluation against L1210 mouse leukemia *in vivo*. Cancer Chemother. Rep., *57:* 141–148, 1973.
6. Houchens, D., Ovejera, A., Johnson, R., Bogden, A., and Neil, G. Therapy of mouse tumors and human tumor xenografts by the antitumor antibiotic AT-125 (NSC-163501). Proc. Am. Assoc. Cancer Res., *19:* 40, 1978.
7. Jayaram, H. N., Cooney, D. A., Ryan, J. A., Neil, G. L., Dion, R. L., and Bono, V. H. L-(αS,5*S*)-α-Amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid (NSC-163501): A new amino acid antibiotic with the properties of an antagonist of L-glutamine. Cancer Chemother. Rep., *59:* 481–491, 1975.
8. Martin, D. G., Duchamp, D. J., and Chidester, C. G. The isolation, structure, and absolute configuration of U-42,126, a novel antitumor antibiotic. Tetrahedron Lett., No. *27:* 2549–2552, 1973.
9. McPartland, R. P., Wang, M. C., Bloch, A., and Weinfeld, H. Cytidine 5′-triphosphate synthetase as a target for inhibition by the antitumor agent 3-deazauridine. Cancer Res., *34:* 3107–3111, 1974.
10. Neil, G. L., Berger, A. E., Hanka, L. J., and Kuentzel, S. L. Biochemical pharmacologic studies with α-amino-3-chloro-2-isoxazoline-5-acetic acid (U-42,126, NSC-163501). A new antitumor antibiotic. *In:* P. Bucalossi, U. Veronesi, and N. Cascinelli (eds.), Abstracts of the Eleventh International Cancer Congress, Florence, Italy, October 20 to 26, 1974, Vol. 4, Panel 69. Milan: Casa Editrice Ambrosiana, 1974.
11. Rustum, Y. M., Creaven, P. J., and Slocum, H. K. Biochemical and pharmacological studies of 3-deazauridine with L1210 cells susceptible and resistant to cytosine arabinoside. Proc. of the 10th International Congress of Chemotherapy Curr. Chemother., 1118–1120, 1978.
12. Savage, C. R., and Weinfeld, H. Purification and properties of mammalian liver cytidine triphosphate synthetase. J. Biol. Chem., *245:* 2529–2535, 1970.
13. Wang, M. C., Sharma, R. A., and Bloch, A. Studies on the mode of action of O^2,2′-anhydrocyclo-1-β-D-arabinofuranosyl cytosine. Cancer Res., *33:* 1265–1271, 1973.