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REVERSAL OF TOXICITY AND ANTITUMOR ACTIVITY OF N-(PHOSPHONACETYL)-L-ASPARTATE BY URIDINE OR CARBAMYL-DL-ASPARTATE IN VIVO

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N-(phosphonacetyl)-L-aspartate (PALA) is a potent transition-state inhibitor of aspartate transcarbamylase (1), and, as such, is an effective inhibitor of de novo pyrimidine nucleotide biosynthesis (2,3). We have recently shown that PALA has antitumor activity in vivo and that its spectrum of activity is unusual, particularly for an antimetabolite (4). PALA is curative in the treatment of mice bearing transplanted Lewis lung carcinoma, a tumor system refractory to most antineoplastic agents including 6-azauridine and pyrazofurin*, inhibitors of de novo pyrimidine nucleotide biosynthesis at the level of orotidylate decarboxylase (5,6). Blockade of de novo pyrimidine nucleotide biosynthesis can be circumvented by utilization of exogenous uridine through the salvage pathway (7). The growth inhibitory effects of PALA in cultured mammalian cells can be reversed by addition of uridine to the culture medium (2). In order to establish that the in vivo antitumor activity of PALA is due to inhibition of aspartate transcarbamylase rather than to some other unknown effect of the drug we attempted to reverse the effects of PALA in tumor-bearing animals by concurrent administration of either uridine or carbamyl aspartate, the product of the aspartate transcarbamylase reaction.

Materials and Methods. PALA was kindly provided by Dr. George R. Stark, Stanford University. Uridine and carbamyl-DL-aspartate were purchased from Sigma Chemical Company (St. Louis, Mo.). PALA and uridine were dissolved in 0.85% NaCl solution for injection. Carbamyl-DL-aspartate was suspended in 0.85% NaCl solution and dissolved by dropwise addition of 5 N NaOH to pH 7.5. Drug solutions were made at an appropriate concentration so that the desired dose was administered in a volume of 0.01 ml/gm body weight. The drugs were administered to male B6D2F$_1$ mice beginning 24 hours after the sc implantation of 10^6 Lewis lung carcinoma cells as previously described (4). PALA was administered ip daily

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for 3 days. Uridine or carbamyl-DL-aspartate was administered sc at 350 mg/kg twice daily (8 AM and 5 PM) for 18 days. On days when both antimetabolite and metabolite were given, PALA was administered immediately prior to the morning injection of the metabolite. Mice were weighed daily and tumors were measured 3 times/week throughout the course of the experiment.

Results. Lewis lung carcinoma grew rapidly in untreated controls. The local tumors grew to a mass of 2 gm in 12.4 days and all mice succumbed to progressive tumor growth with a mean survival time of 24.9 days (Table 1). Administration of either uridine or carbamyl-DL-aspartate alone gave no evidence of toxicity and had no significant influence on tumor growth or survival of tumor-bearing mice.

Table 1. Effects of carbamyl-DL-aspartate and uridine on the toxicity and antitumor activity of PALA in $B6D2F_1$ mice bearing sc Lewis lung carcinoma

PALA mg/kg/day, ip, days 1-3	Metabolite 350 mg/kg, bid, sc, days 1-18	Toxicity: Max. wt loss (%)	Toxicity: Toxic deaths (total)	Antitumor Activity[a]: Time to reach 2 gm (days)[b]	Antitumor Activity[a]: Lifespan (days)[b]	Antitumor Activity[a]: Cures[c] (total)
Untreated Control		4.0	0/10	12.4±1.5	24.9±4.9	0/10
-	Carbamyl-DL-aspartate	6.8	0/10	12.5±2.8	23.0±4.8	0/10
	Uridine	8.6	0/10	12.6±1.7	27.1±7.7	0/10
880	-	21.0[d]	10/10	toxic	toxic	toxic
880	Carbamyl-DL-aspartate	19.2[d]	10/10	toxic	toxic	toxic
880	Uridine	25.8	5/10	23.7±2.3[e]	40.6±15.0[e]	0/5
528	-	27.0	4/10	>73[e]	>73[e]	4/6
528	Carbamyl-DL-aspartate	12.0	6/10	15.0±1.9	31.2±6.3	0/4
528	Uridine	16.0	0/10	18.8±2.1[e]	34.5±5.9[e]	0/10
317	-	18.0	2/10	>73[e]	>73[e]	6/8
317	Carbamyl-DL-aspartate	10.6	0/9	15.6±3.2	27.4±3.8	0/9
317	Uridine	12.8	0/10	18.8±2.7[e]	32.8±6.3[e]	0/10

[a]Excludes toxic deaths. [b]Mean ± S.D. [c]Tumor-free survivors on day 73. [d]Weight loss at the time of death. [e]Significantly different from untreated control ($p<0.01$).

The highest dose of PALA, 880 mg/kg on days 1-3, was lethal when administered alone; the mice succumbed within 7 days with progressive weight loss. The administration of carbamyl-DL-aspartate with this lethal dose of PALA did not protect against the toxicity. On the other hand, uridine administration protected half of the animals from the lethal effects of this dose of PALA, even though weight loss parallelled that of mice treated with 880 mg/kg of PALA alone. Tumors grew and killed the 5 mice which did not die from toxicity following treatment with uridine plus the highest dose of PALA. There was, however, a significant delay in tumor growth and a corresponding increase in survival time.

PALA at 528 mg/kg caused extensive weight loss and was lethal to 4 of 10 mice. Two of the 6 remaining animals developed tumors and died on days 45 and 50; 4 mice were sacrificed on day 73 and showed no evidence of local or metastatic tumor. Carbamyl-DL-aspartate decreased the weight loss but did not protect from the lethal toxicity of this dose of PALA. However, there was a complete block of the effects of PALA on the tumor. Uridine administration prevented the lethal toxicity of 528 mg/kg of PALA and also reversed the antitumor activity as all animals succumbed to progressive tumor growth.

The lowest dose of PALA (317 mg/kg, daily for 3 days) was lethal to only 2 of 10 mice and cured 6 of the 8 remaining animals. Both carbamyl-DL-aspartate and uridine prevented both the toxicity and antitumor activity of this dose of PALA.

Discussion. The finding that carbamyl-DL-aspartate can completely reverse the effects of PALA on Lewis lung carcinoma provides strong evidence that the antitumor activity of this agent is due to its inhibition of aspartate transcarbamylase, as carbamyl-L-aspartate is the end product of this enzymatic reaction. Reversal of the toxic and antineoplastic effects of PALA by uridine is in agreement with studies in tissue culture which showed that the growth inhibitory activity of PALA was antagonized by uridine (2). The effects of uridine also correspond to the alleviation of symptoms of hereditary orotic aciduria (8) and prevention of toxicity of pyrazofurin (9) by administration of this nucleoside.

Effective tissue levels of PALA are maintained for long periods (3) and were reflected in this experiment by a weight loss of greater than 10 percent which persisted for 20 days after treatment with PALA at 528 or 317 mg/kg/day x 3. This prolonged weight loss prompted the continued administration of the metabolites for 18 days. Animals treated with the lower two doses of PALA plus either of the metabolites regained their pretreatment weights by day 12. It is possible that higher doses or infusions of the metabolites would have been more effective in preventing the toxicity and antitumor activity of PALA.

In this study it appeared that carbamyl-DL-aspartate was more effective in blocking the antitumor activity of PALA than in preventing toxicity to the host, whereas uridine prevented toxicity to a greater degree without completely reversing the antitumor activity as there was a statistically significant delay in tumor growth and prolongation of survival in animals treated with PALA plus uridine. Selective protection of the host with retention of antitumor activity has been obtained with appropriate timing of leucovorin administration following antifol therapy (10). Whether selective protection from PALA toxicity can be obtained will be the subject of further studies in which dose level and timing of administration of uridine, carbamyl aspartate, and other potential protective agents, such as orotate or dihydroorotate, will be manipulated.

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References

1. K.D. Collins and G.R. Stark, J. Biol. Chem. 246, 6599 (1971).
2. E.A. Swyryd, S.S. Seaver and G.R. Stark, J. Biol. Chem. 249, 6945 (1974).
3. T. Yoshida, G.R. Stark and N.J. Hoogenraad, J. Biol. Chem. 249, 6951 (1974).
4. R.K. Johnson, T. Inouye, A. Goldin and G.R. Stark, Cancer Res. 36, 2720 (1976).
5. R.E. Handschumacher and C.A. Pasternak, Biochim. Biophys. Acta 30, 451 (1958).
6. M.J. Sweeney, G.E. Gutowski, G.A. Poore and R.L. Hamill, Proc. Amer. Assoc. Cancer Res. 13, 108 (1972).
7. R.W. Brockman and E.P. Anderson, Ann. Rev. Biochem. 32, 463 (1963).
8. L.H. Smith, Jr., C.M. Huguley, Jr. and J.A. Bain, in The Metabolic Basis of Inherited Disease (Eds. J.B. Stanbury, J.B. Wyngaarden and D.S. Frederickson) p. 739. McGraw-Hill, New York (1966).
9. E.C. Cadman, D.E. Dix, S.C. Hill and R.E. Handschumacher, Proc. Amer. Assoc. Cancer Res. 17, 208 (1976).
10. A. Goldin, J.M. Venditti, S.R. Humphreys, D. Dennis, N. Mantel and S.W. Greenhouse, Cancer Res. 15, 57 (1955).