Mechanism of Synergy between N-Phosphonacetyl-L-aspartate and Dipyridamole in a Human Ovarian Carcinoma Cell Line

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ABSTRACT

Previous results from our laboratory have shown that the nucleoside transport inhibitor dipyridamole (DP) markedly augmented both the in vitro and in vivo activities of the pyrimidine antimetabolite N-phosphonacetyl-L-aspartate (PALA). In a human ovarian carcinoma cell line (2008), DP increased the activity of PALA by 1 to 2 logs in growth rate and clonogenic assays while exhibiting no cytotoxicity of its own. The concentration of DP used (1 |lM) in these assays resulted in over 80% reduction in uridine uptake in the 2008 cells at the end of 1 h. The activity of PALA and PALA plus DP was completely antagonized by the addition of exogenous uridine in a dose-dependent manner. Addition of other nucleosides to concentrations as high as 1000 |lM failed to rescue the ovarian cells from the drug combination, and combining two nucleosides together did not antagonize PALA and PALA plus DP activity to any greater extent. Cellular nucleotide pool analysis by anion-exchange high-performance liquid chromatography revealed that dipyridamole further reduced the already depressed uridine triphosphate and cytidine triphosphate pools of cells exposed to PALA, while the guanosine triphosphate pool was slightly elevated. Uridine supplementation resulted in partial replenishment of the uridine triphosphate and cytidine triphosphate pools, but the absolute levels remained below control values. The acute drug-induced changes in nucleotide pools in 2008 xenografts growing in athymic mice paralleled those observed in vitro. Evidence presented here supports the ability of DP to potentiate PALA activity against a human ovarian carcinoma cell line. The mechanism of synergy relates to the inhibition of pyrimidine salvage in the tumor cells via the blockade of uridine uptake.

INTRODUCTION

PALA3 is a stable transition-state analogue inhibitor of ATCase, an enzyme in the de novo pyrimidine-biosynthetic pathway. PALA has an affinity for Escherichia coli ATCase which is 1000 times greater than that of the natural substrate carbamyl phosphate (2), and the drug is a potent inhibitor of pyrimidine biosynthesis in mammalian cells (3). It markedly depresses mouse liver ATCase activity within 15 min of administration, and the effect persists beyond 72 h after a single dose (4). PALA has a unique antitumor spectrum in mice, with the rapidly proliferating murine leukemias being resistant, and the slower-growing Lewis lung carcinoma and B16 melanoma being sensitive to the drug (5, 6). The uniqueness of the activity of PALA and lack of myelosuppression in preclinical studies (6–8) made this drug a good candidate in human cancer trials. Unfortunately, several Phase I (9–14) and Phase II non-small cell lung cancer (15, 16), colon cancer (17–19), malignant melanoma (20), and head and neck cancer (21) trials have failed to reveal significant activity of this drug against any of the tumors evaluated.

Several mechanisms have been proposed to explain the lack of efficacy of PALA against human tumors in vivo. (a) ATCase is not the rate-limiting enzyme in de novo pyrimidine synthesis (22); therefore, its activity must be markedly inhibited before the rate of pyrimidine synthesis is depressed. This probably explains the necessary but not sufficient requirement of low ATCase activity in the tumor for there to be any response to the PALA (23). (b) It has been demonstrated in Lewis lung carcinoma lines made resistant to PALA that the activity of carbamyl phosphate synthetase is increased (24). It is possible that the resultant increase of carbamyl phosphate can successfully compete with PALA for ATCase, allowing de novo pyrimidine synthesis to proceed despite the presence of the drug (24). (c) It is possible that even with complete inhibition of ATCase activity by PALA, pyrimidine synthesis proceeds normally via the "salvage pathway" by reutilization of uridine and/or cytidine (22, 25). It has been established in experimental systems that exogenous uridine can reverse both the toxic and antitumor effects of PALA (3, 26, 27).

If it were possible to prevent tumor cells from salvaging uridine when de novo pyrimidine synthesis is inhibited by PALA, greater antitumor activity might be achieved. One such approach would be to block uridine transport in tumor cells. DP, a drug used clinically as a vasodilator and ant platelet agent, is a potent inhibitor of membrane uridine transport (28, 29). The exact mechanism of its action and the chemical basis for its high affinity for the transport site on cell membranes is still largely unknown. Our interest in DP as a potential modulator for the cytotoxic activity of antimitabolites stems from its use in thousands of patients; it has already established its clinical pharmacology and its lack of serious toxicity in humans. We have reported earlier that, at a nontoxic and relevant concentration (1 |lM), DP markedly augmented the cytotoxicity of PALA against several human tumor cell lines in soft-agar clonogenic assays. DP also produced 50% inhibition of radiolabeled uridine uptake in these same cells at concentrations of less than 0.1 |lM. Toxicity studies in mice showed that DP can reduce the 50% lethal dose of PALA approximately 2-fold in these animals (30). The study reported here demonstrated that the mechanism of synergy between DP and PALA is related to more extensive depletion of intracellular pyrimidine nucleotides produced by the combination than by either drug alone.
MATERIALS AND METHODS

Drugs and Reagents. PALA (100 mg/ml) was obtained from the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD), and DP (Persantine, 5 mg/ml) was supplied by Boehringer Ingelheim, Ltd. (Ridgefield, CT). All nucleosides and nucleotide standards were purchased from Sigma Chemical Co. (St. Louis, MO), and all other chemicals used were obtained from Fisher Scientific (Fairlawn, NJ). PBS was purchased from Oxoid Ltd., England.

Cell Line. A human ovarian carcinoma cell line 2008 (31) was used in all of the experiments. Cells were maintained in logarithmic growth in RPMI 1640 supplemented with 10% fetal bovine serum and 1% L-glutamine.

Growth Assay. Samples of 2008 cells growing in monolayer in T-75 flasks (Falcon Plastics, Cockeysville, MD) were harvested after trypsin/EDTA treatment. They were washed twice in fresh medium and seeded at a cell concentration of 2 x 10^6/ml into Linbro multiwell culture plates (Flow Laboratories, McLean, VA) with varying concentrations of drugs and nucleosides already pipetted into the wells in triplicate. The plates were incubated under 5% CO2 at 37°C for 72 h, and the cell number in each well was quantified with an electronic cell counter (Coulter Electronics, Hialeah, FL) after trypsinization.

Clonogenic Assay. Cells growing in log phase were harvested, washed, dispersed, and plated onto 60-mm plastic Petri dishes (Coming Glass Works, NY) in triplicate at a density of 400 cells/dish. Varying amounts of drugs and nucleosides were added to the dishes, and the cells were incubated under 7% CO2 at 37°C for 10 days. Clusters of >50 cells were counted as one colony, and usually the control dishes contained 100 to 120 colonies.

Uridine Uptake in 2008 Cells. Freshly harvested 2008 cells were suspended in fresh medium with or without DP for 5 min at 37°C under a 5% CO2 atmosphere. [5,6-3H]Uridine (23.4 Ci/mmol; New England Nuclear, Boston, MA) at a final concentration of 10 ¿M was added, and the suspension was vortexed rigorously. Aliquots of the cell suspension (1 x 10^6 cells for each data point) were removed at 5, 10, 15, 20, 30, and 60 min and diluted into 10 volumes of ice-cold PBS. The samples were centrifuged at 4°C for 5 min at 500 x g, and the supernatants were discarded. The cell pellets were washed twice with 1 ml of cold PBS and then resuspended in 900 ¿l of 0.1 N NaOH and maintained on ice for 10 min. A 500-¿l aliquot of each resulting sample was then suspended in 10 ml of ScintVerse Bio-HP (Fisher Scientific), and the radioactivity in each well was quantified by liquid scintillation counting.

Nucleoside Measurements. The concentrations of nucleosides in the culture media were confirmed using a modified reverse-phase HPLC method developed in this laboratory (32). Briefly, aliquots of media were taken at various time points of incubation and deproteinized by centrifugation (2000 x g) through Amicon Centriflo CF-25 Ultrafiltration membrane cones (Amicon Corp., Danvers, MA). All nucleosides of interest were recovered completely from this procedure. A carefully measured volume of the ultrafiltrate was injected into the HPLC column, and the absorbance at 254 nm was measured for nucleoside quantitation. The HPLC system consisted of the following Waters Associates equipment: one Model 6000A pump; Model 440 UV absorbance detector; Model U6K injector; Z-module radial compression unit; and Model 730 data analysis system. Two pumps effluents were monitored by Waters alloy-exchange radial compression cartridge in a Z-module unit. The buffers were: Solution A, 0.1 M KH2PO4, pH 4.0; and Solution B, 0.25 M KH2PO4/0.5 M KCl, pH 5.0. The initial conditions were 80% Solution A and 20% Solution B, and triphosphate nucleotides were eluted with a hyperbolic solvent gradient (Curve 4 of the Waters Model 600 solvent program) generated over 30 min to 100% Solution B. Nucleotide identity was confirmed by 254/280 nm absorbance ratios and coelution of cellular extracts with nucleotide standards. The time courses of nucleotide pool changes under various drug treatments were also studied. Aliquots of cell suspension were removed hourly up to 24 h following their exposure to PALA and PALA plus DP (same concentrations as in Table 2), and their cellular nucleotide levels were assayed as outlined above. Nucleotide levels were calculated from absorbance at 254 nm and extrapolated from standard curves constructed from a solution of mixed nucleotide standards eluted under the same conditions. Values were normalized to pmol/million cells.

Tissue Nucleotide Levels. Athymic mice were given s.c. injections of 5 x 10^6 viable 2008 cells. The resultant tumors were passaged continuously, and animals with initial tumor volumes between 0.25 and 0.50 ml were used in the study. PALA was given i.p. at a dose of 200 mg/kg, and DP was given s.c. at a dose of 100 mg/kg. Sterile 0.9% NaCI solution was given via the same routes in control animals. At 24 and 48 h posttreatment, the mice were sacrificed by cervical dislocation, and the tumors were excised rapidly and plunged into liquid nitrogen. After the tumor weights were recorded, the tissues were pulverized into a fine powder under liquid nitrogen and extracted with aliquots of 0.6 N perchloric acid. The acid extracts were neutralized with 2.2 M potassium bicarbonate and centrifuged, and the supernatants were used for HPLC analysis of the nucleotide pool as described above. Nucleotide levels were expressed as pmol/mg of tumor tissue.

RESULTS

PALA and DP Synergy. In growth rate assays (Chart 1A), DP (1 ¿M) was nontoxic to 2008 cells and PALA was only moderately cytotoxic, with a decrease in growth rate of approximately 40% at a concentration of 500 ¿M. The addition of 1 ¿M DP enhanced the cytotoxicity of PALA at all concentrations, with a decrease in growth rate now greater than 90% at 500 ¿M PALA. Qualitatively similar results were observed in the clonogenic assays (Chart 1B) where 1 ¿M DP resulted in less than 5% reduction in colony formation, and 50 ¿M PALA alone inhibited colony formation to 80% of control. The combination of 1 ¿M DP with different concentrations of PALA, however, resulted in much increased inhibition of colony formation, and complete inhibition was effected at 50 ¿M PALA.

Uridine Uptake. The cellular uptake of radiolabeled uridine by the 2008 cells was measured in the absence and in the presence of different concentrations of DP. The rates of radioactive uridine incorporation into 10^6 cells at various time points in a typical experiment are shown in Chart 2. In the control cells, the rate of uptake is essentially linear during the first 30 min. In our experiments, even 0.1 ¿M DP produced marked inhibition of uridine uptake, and 1 ¿M DP exhibited maximal or near-maximal inhibition in these cells. The slopes of radioactive incorporation in 2008 cells from 5 experiments were calculated using least square regression; the value for control cells was 153 ± 42 (SD), and that for cells treated with 1 ¿M DP was 25 ± 11. The slopes were highly significantly different by the one-tailed t test (P <
Chart 1. In vitro cytotoxic activity of PALA and PALA plus DP. A, results of growth rate assays of 2008 cells in the presence of increasing concentrations of PALA with and without 1 µM DP. Each point represents the mean of 5 experiments; bars, SD. All values above 10 µM PALA were significantly different between DP-treated and untreated cells (P < 0.01). B, results of clonogenic assays on plastic plates. After 10 days of incubation, clusters of cells greater than 50 were counted as one colony. Each point represents the mean of 5 experiments; bars, SD. DP alone caused a 5% reduction in colony formation. All the values in the presence of DP were significantly different from those in the absence of DP at all PALA concentrations (P < 0.01).

When the data were replotted as the amount of radioincorporation after 30 min of incubation in the presence of varying concentrations of DP, the apparent 50% inhibitory concentration for uridine uptake was calculated to be in the range of 0.02 to 0.07 µM DP in these cells.

Nucleoside Rescue. The ability of nucleosides to antagonize the in vitro activity of PALA was explored by subjecting the cells to cytotoxic concentrations of PALA and PALA plus DP in the presence of increasing amounts of thymidine, adenosine, guanosine, cytidine, and uridine. Normally, there are low levels (between 1 and 2 µM) of uridine and cytidine in the freshly prepared growth medium. Of the 5 tested, the only nucleoside that antagonized PALA and PALA/DP cytotoxicity was uridine. In clonogenic assays, the colony formation of both PALA- and PALA/DP-treated cells responded in a dose-dependent fashion to added uridine. The data in Chart 3 indicate that the effects of 50 µM PALA alone were completely reversed by 100 µM exogenous uridine, while antagonism of 50 µM PALA/1 µM DP required 500 µM uridine. Thymidine by itself began to show cytotoxicity at 100 µM and reduced the colony counts to 20% of control at 1 mM. Instead of rescuing the cells, thymidine enhanced the toxicity of PALA and PALA/DP in parallel to its own toxicity at any given concentration (data not shown). In a series of growth rate assays, the addition of the other nucleosides singly (up to 1 mM) did not affect PALA/DP cytotoxicity, and combinations of nucleosides did not result in greater antagonism of PALA/DP toxicity (Table 1).

Chart 2. Inhibition of radioactive uridine uptake into 2008 cells by DP. Cells were suspended in fresh medium containing the various concentrations of DP. [5,6-³H]uridine was added to each suspension to make up the final concentration of 10 µM uridine, and aliquots of cell suspension were removed at timed intervals, washed, and digested with 0.1 N NaOH. The amount of radioactivity in each sample was quantified with liquid scintillation counting. The uptake is essentially linear for the first 30 min, and 1 µM DP inhibited over 80% of the uridine uptake in the 2008 cells.

Chart 3. Rescue of 2008 cells treated with PALA and PALA plus DP by exogenous uridine. Clonogenic assays of PALA- and PALA/DP-treated cells in the presence of increasing concentrations of uridine were set up as described in "Materials and Methods." Each point represents the mean of 5 experiments; bars, SD.
Table 1

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cell counts (as % of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>PALA</td>
<td>62</td>
</tr>
<tr>
<td>PALA/DP</td>
<td>16</td>
</tr>
<tr>
<td>+ uridine (0.1 mM)</td>
<td>57</td>
</tr>
<tr>
<td>+ uridine (1 mM)</td>
<td>99</td>
</tr>
<tr>
<td>+ cytidine (1 mM)</td>
<td>15</td>
</tr>
<tr>
<td>+ guanosine (1 mM)</td>
<td>17</td>
</tr>
<tr>
<td>+ adenosine (1 mM)</td>
<td>15</td>
</tr>
<tr>
<td>+ adenosine/guanosine (0.1 mM)</td>
<td>18</td>
</tr>
<tr>
<td>+ adenosine/cytidine (0.1 mM)</td>
<td>13</td>
</tr>
<tr>
<td>+ guanosine/cytidine (0.1 mM)</td>
<td>14</td>
</tr>
<tr>
<td>+ cytosine/uridine (0.1 mM)</td>
<td>57</td>
</tr>
</tbody>
</table>

This is the result of a typical experiment. Each value is the mean of triplicates where the cell counts for each data point are usually within 5% of each other.

DISCUSSION

In a human ovarian carcinoma cell line, we have confirmed that DP, while relatively nontoxic by itself, augmented the cytotoxicity of PALA at all drug concentrations. Since uridine is the only nucleoside that fully antagonized both PALA and PALA/DP toxicity in a dose-dependent manner and the concentration of DP used in these experiments inhibited over 80% of uridine uptake acutely in the 2008 cells, the results suggest that the effects of this drug combination are mediated specifically through uridine metabolism. Cellular and tissue nucleotide levels confirmed that PALA/DP treatment inhibited the biosynthesis of pyrimidine nucleotides more severely than did PALA alone, and the resultant UTP and CTP deficit was associated with very low viability in the treated cells. To obtain further evidence that DP worked via the blockade of nucleoside salvage, we have attempted to duplicate the above experiments in RPMI 1640
### Table 2

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Nucleotides (pmol/10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UTP</td>
</tr>
<tr>
<td>1. Control</td>
<td>159 ± 15</td>
</tr>
<tr>
<td>2. DP (1 μM)</td>
<td>186 ± 32</td>
</tr>
<tr>
<td>3. PALA (50 μM)</td>
<td>25 ± 90</td>
</tr>
<tr>
<td>4. PALA (50 μM) + DP (1 μM)</td>
<td>B/D</td>
</tr>
<tr>
<td>5. PALA + DP + uridine (500 μM)</td>
<td>112 ± 24</td>
</tr>
<tr>
<td>6. Uridine (500 μM)</td>
<td>270 ± 71</td>
</tr>
</tbody>
</table>

* Mean ±SD of 6 experiments; measured after 72 h of incubation.  
* Significantly different when compared to the corresponding values of the control group (P < 0.05, one-tail paired t test). When comparisons between the various treatment groups were made, statistical significance was ascertained using one-way analysis of variance and Duncan's multiple-range test with the same level of significance chosen (see text for results).  
* B/D, below the limit of detection, approximately 15 pmol/10⁶ cells.

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### Table 3

<table>
<thead>
<tr>
<th>Nucleotides</th>
<th>Control</th>
<th>DP (100 mg/kg)</th>
<th>PALA (200 mg/kg)</th>
<th>PALA (200 mg/kg) + DP (100 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UTP</td>
<td>155 ± 18</td>
<td>162 ± 38</td>
<td>74 ± 22</td>
<td>B/D</td>
</tr>
<tr>
<td>CTP</td>
<td>62 ± 17</td>
<td>69 ± 13</td>
<td>49 ± 8</td>
<td>B/D</td>
</tr>
<tr>
<td>ATP</td>
<td>689 ± 48</td>
<td>681 ± 54</td>
<td>723 ± 59</td>
<td>636 ± 65</td>
</tr>
<tr>
<td>GTP</td>
<td>365 ± 41</td>
<td>333 ± 49</td>
<td>285 ± 44</td>
<td>327 ± 31</td>
</tr>
<tr>
<td>24 h (n = 4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UTP</td>
<td>167 ± 29</td>
<td>158 ± 43</td>
<td>78 ± 25</td>
<td>B/D</td>
</tr>
<tr>
<td>CTP</td>
<td>71 ± 22</td>
<td>74 ± 11</td>
<td>54 ± 9</td>
<td>B/D</td>
</tr>
<tr>
<td>ATP</td>
<td>674 ± 55</td>
<td>683 ± 48</td>
<td>654 ± 84</td>
<td>642 ± 73</td>
</tr>
<tr>
<td>GTP</td>
<td>343 ± 38</td>
<td>361 ± 47</td>
<td>369 ± 52</td>
<td>377 ± 42</td>
</tr>
<tr>
<td>48 h (n = 3)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* Mean ±SD.  
* Significantly different when compared to the corresponding control values (P < 0.05, Student's t test).  
* B/D, below the limit of detection, approximately 40 pmol/mg.

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weight growth factors were removed by the dialysis or adsorption procedures, because the reverse-phase HPLC profiles of the treated culture medium (not shown) looked very different from that of the fresh medium.

DP treatment alone reduced all 4 triphosphate nucleotide levels by an average of 30% in the 2008 cells. This suggests that the de novo synthetic mechanisms surprisingly did not increase their rates enough to fully make up the deficit produced by the inhibition by DP of the salvage pathway. One possible but unlikely explanation is that this observation is a peculiar characteristic of the 2008 cells. Another more likely explanation is that, under normal (basal) conditions, the cells rely heavily on the salvage pathway for nucleotide biosynthesis and the sudden blockade of this mechanism required major adjustments of the de novo mechanisms that take longer than 72 h to fully make up for the deficit. There is some evidence supporting this line of argument in the 3924A hepatoma line where the salvage enzymes seem to have higher basal activity than do the de novo enzymes (33). Finally, the possibility that DP has other effects on cell membranes and nucleotide metabolism that affect the ability of the de novo mechanisms to make up for the defect should not be discounted. There is evidence that, in addition to its ability to inhibit membrane nucleoside transport, DP can block the uptake of glucose and inorganic phosphates as well (34). The blockade of either or both pathways may result in a decrease in cellular

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supplemented with dialyzed or charcoal/dextran-treated fetal bovine serum (to remove endogenous nucleosides). The 2008 cells unfortunately stopped growing in the new medium shortly after subculturing. It is likely that some essential low-molecular-weight growth factors were removed by the dialysis or adsorption procedures, because the reverse-phase HPLC profiles of the treated culture medium (not shown) looked very different from that of the fresh medium.

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de novo nucleotide synthesis leading to the depressed levels observed in these studies.

PALA treatment alone resulted in marked decreases in the pyrimidine nucleotide levels (over 90% for UTP and 60% for CTP), and yet it was only associated with moderate cell kill (20 to 40%) in both the growth and clonogenic assays. Our results qualitatively confirmed the earlier work of Moyer and Handschumacher (7) who reported profound UTP and CTP depletion in L1210 and Lewis lung cells treated with comparable concentrations of PALA, but the cytotoxicity did not correlate well with nucleotide pool changes in different cell lines. Taken together with the DP effects on nucleotide levels, this apparent paradox confirms that the drug-induced disruption in the absolute levels of nucleotides within a cell is a poor predictor of cytotoxicity. Uziel and Selikirk's (35) proposal that nucleotides are probably compartmentalized within cells offers a partial explanation for this observation. Another possible explanation was offered by Moyer et al. (36), who proposed that the mechanism of PALA toxicity may reside in the disruption of deoxyribonucleotide pools and that the inhibition of DNA rather than RNA synthesis is responsible for PALA toxicity.

PALA and DP together produced further depression of the UTP and CTP pools to below detection and concurrently decreased the ATP level when compared to cells treated with PALA alone (P < 0.05, ANOVA). This regimen corresponds to a greater than 90% cell kill in the clonogenic assays. Interestingly, the GTP pool is slightly elevated in the PALA-treated cells when compared to control (P < 0.1, paired t test), and a similar elevation is seen in the PALA/DP-treated cells where the GTP level was marginally higher than in cells treated with DP alone (P < 0.1, ANOVA). These observations suggest that the relative ratios of the nucleotides as well as their concentrations are important in determining cell viability. These results also suggest the possibility of "cross-talk" between the purine and pyrimidine systems within the cell and that purine metabolism may play a role in PALA/DP toxicity. Uridine supplementation of PALA/DP-treated cells resulted only in partial replenishment of the UTP, CTP, and ATP pools when compared to control cells, and yet cell growth was similar to that of untreated cells. This observation further emphasizes the discrepancy between the absolute nucleotide levels and cell viability.

The 2008 cells were used in these experiments because of their ability to form well-defined colonies on plastic and their relatively short doubling times. The large size of these cells also makes them convenient for nucleotide extraction and measurements. These cells also proliferate very well as xenografts in athymic mice. The data on nucleotide levels within tumor xenografts qualitatively confirmed the findings in tissue culture except that DP alone did not produce any reduction of tissue triphosphate nucleotides. A possible explanation is that DP is so highly protein bound in vivo (37) that the concentration achieved at the tumor site is too low to exhibit its other effects. The reported range of plasma uridine levels in cancer patients (25, 32, 38, 39) is between 3 and 10 μM, a concentration range where uridine antimetabolite toxicity became measurable in our experiments. It is possible that uridine salvage plays a role in conferring PALA resistance in cancer patients. Zhen et al. (40) reported that 5 μM DP did not affect the cytotoxicity of acivicin (a glutamine antagonist) against a hepatoma line but inhibited the protective actions of exogenous nucleosides. Fischer et al. (41) reported that DP at this concentration inhibited the repletion of CTP and GTP pools in colon cancer cells and proposed that DP restored the antiproliferative activity of acivicin via the blockade of the salvage pathways. These observations tend to support a clinical role for DP in new antineoplastic regimens. Since PALA is a drug associated with relatively few toxic side effects and minimal myelosuppression, any regimen that can improve its activity in vivo may have important clinical implications. DP, a drug that has been used safely for over 2 decades, may confer efficacy and perhaps selectivity to antimitabolites such as PALA.

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PALA AND DP SYNERGY


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