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Dipyridamole Mediated Enhanced Antiproliferative Activity of 10-Ethyl-10-Deazaaminopterin (10-EDAM) Against Human Lung Cancer Cell Lines

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Abstract 10-ethyl-10-deazaaminopterin (10-EDAM) is a rationally designed derivative of the antifolate, methotrexate (MTX). In a number of tumor models these design features have resulted in an improved spectrum of antiproliferative activity as compared with the parent compound. Using an MTT growth assay, we compared in vitro antiproliferative activity of 10-EDAM with MTX in eight lung cancer cell lines. Growth was inhibited in all lines tested by clinically achievable concentrations of 10-EDAM (0.1–1,000 nM). 10-EDAM was more cytotoxic than MTX at the same concentrations in all eight lung cancer cell lines. In an effort to enhance the antiproliferative effect, we evaluated the addition of dipyridamole (DPM), an inhibitor of nucleoside transport, to 10-EDAM (0.1–10 μm). DPM decreased the concentration of 10-EDAM required to cause 50% growth inhibition (IC_{50}) in all eight cell lines tested. This suppression was statistically significant by 2-sided sign test ($P = .0078$). By contrast, the IC_{50} of MTX was decreased *in only two of* the eight cell lines when DPM was added (0.1–10 μM). In defined thymidine depleted media, cell kill by the combination of 10-EDAM and DPM was no greater than 10-EDAM alone, consistent with the possibility that DPM exerts some of its effect by inhibition of extrinsic nucleoside salvage. In consideration of the published activity of 10-EDAM in lung cancer and the modest clinical toxicity of DPM based biochemical modulation, we conclude the current in vitro data provide justification for clinical evaluation of this combination in patients with lung cancer. © 1996 Wiley-Liss, Inc.*

Key words: 10-EDAM, dipyridamole, methotrexate, lung cancer

The antifolate, 10-ethyl-10-deazaaminopterin (10-EDAM, edatrexate), is an analog of methotrexate (MTX) synthesized by modification of the N^{10} position of 4-aminofolate [1] (Fig. 1). When compared to MTX, 10-EDAM has greater cytotoxic activity against a number of murine ascites and solid tumors [2] and human tumor xenografts (MX-1, LX-1, CX-1) [3]. This analog differs from MTX both in regard to lipophilicity, and as a substrate for intracellular polyglutamation. Modification of the N^{10} position of 4-aminofolate (Fig. 1) results in a significant change in membrane transport and polglutamation between a variety of tumor types as compared to normal proliferative tissues [1,2]. Moreover, it appears 10-EDAM potentially has an improved therapeutic index when compared to MTX, possibly related to its enhanced entry and retention within tumor cells, along with its relative rapid clearance and exclusion from sensitive host tissues such as the small intestine [1–4]. In murine tumor models, 10-EDAM displayed superior antitumor activity as compared to 10-deazaaminopterin, the initial analog in this series of N^{10} substituted compounds. This increased activity was correlated with higher concentrations of polyglutamate forms of 10-EDAM than 10-deazaaminopterin in tumor cells [1].

Abbreviations used: DHFR, dihydrofolate reductase; DPM, dipyridamole; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; MTX, methotrexate; NSCLC, non-small cell lung cancer; PALA, N-phosphonacetyl-l-aspartate; SCLC, small cell lung cancer; TS, thymidylate synthase; 10-EDAM, 10-ethyl-10-deazaaminopterin.

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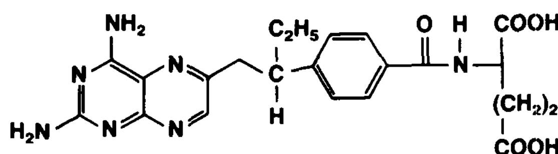
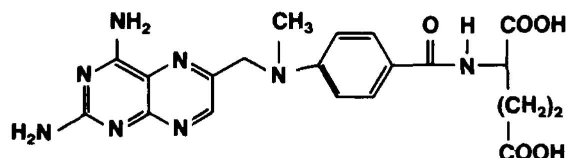
Because of its significant activity in various tumor model systems, 10-EDAM was quickly advanced to clinical trials [5–7]. In a phase I study, partial tumor regressions were noted in 3 of 36 (8%) non-small cell lung cancer (NSCLC) patients [5], and, in a follow-up study, 6 of 19 (32%) patients demonstrated a response in a single agent phase II study (95% CI = 11–52%). Toxicity was qualitatively similar to that of MTX [6]. A phase II clinical trial followed in which 10-EDAM was combined with mitomycin and vinblastine in the treatment of NSCLC [7]. Of thirty evaluable patients, a response rate of 60% was observed (95% CI = 42–78%). A phase III study evaluating mitomycin and vinblastine, with or without 10-EDAM, is in progress to evaluate the role of 10-EDAM in this two drug regimen. A recent phase II study of 10-EDAM in both previously treated and untreated patients with extensive stage small cell lung cancer (SCLC) produced less encouraging results [8]. No clinically significant activity of this agent was seen in this group of 33 patients receiving 10-EDAM at five different dose levels. A phase III randomized study in head and neck cancer comparing the efficacy of methotrexate to 10-EDAM showed demonstratable activity of 10-EDAM in this disease with a 16% response rate [9]. However, 10-EDAM was not superior to methotrexate in this study and had greater toxicity. In summary, although 10-EDAM has shown promise in a variety of solid tumors, it remains unclear if it will have a significant role in the treatment of these diseases. Of the malignancies in which 10-EDAM has been tested, NSCLC is the most promising and remains an area of continuing investigation [10,11]. Recent studies have investigated the role of biochemical modulation in regimens combining edatrexate and other agents to increase the efficacy of this new agent in lung cancer [12].

Dipyridamole (DPM) is an established antithrombotic agent as well as a coronary vessel dilator [13]. Although this drug has very little antitumor activity on its own, there have been numerous published reports of its ability to enhance the cytotoxic effects of other antineoplastic agents. These include 5-fluorouracil [14,15], ara-c [16], MTX [17,18], PALA [19], etoposide [20,21], vinblastine [21], and doxorubicin [21,22]. DPM has been shown to increase drug influx (vinblastine), decrease drug efflux (vinblastine, etoposide, and doxorubicin), and inhibit nucleoside salvage (5-fluorouracil, MTX, ara-c) [14–22].

10-Ethyl-10-deaza-aminopterin (10-EDAM)



METHOTREXATE (MTX)



DIPYRIDAMOLE (DPM)

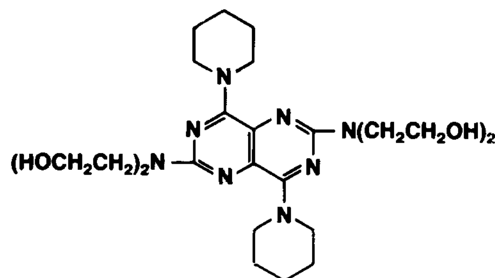


Fig. 1. Chemical structures of 10-EDAM, MTX, and DPM. The structures of 10-EDAM, MTX, and DPM are depicted as indicated.

The present study was undertaken to compare the in vitro antiproliferative activity of 10-EDAM to MTX against a panel of human lung cancer cell lines. We further determined the ability of DPM to modulate the antiproliferative activity of 10-EDAM in vitro against human lung cancer cell lines using clinically achievable concentrations. Given the established activity of 10-EDAM in human lung cancer, our data suggests there is a rational basis to believe the addition of DPM in this patient population may result in enhanced antitumor effect without significantly increasing the toxicity. Given the lack of success with traditional dose escalation strategies in this disease [23] biochemical modulations are another type of strategy which merit serious consideration [24].

MATERIALS AND METHODS

Cell Lines

The lung cancer cell lines used were NCI-H23, NCI-H146, NCI-H157, NCI-H187, NCI-H322,

TABLE I. Lung Cancer Cell Lines*

Cell line	Prior therapy	Histology	Tissue source
SCLC			
NCI-H146	Chemotherapy	Classic	Pleural effusion
NCI-H187	None	Classic	Pleural effusion
NCI-N417	None	Variant	Lung biopsy
NSCLC			
NCI-H23	None	Adenocarcinoma	Lung biopsy
NCI-H157	None	Squamous	Pleural effusion
NCI-H322	None	BAC	Lymph node
NCI-H460	None	Large cell	Pleural effusion
NCI-H1299	None	Large cell	Lymph node

*The lung cancer cell lines used are as shown. Histology was classified as classic or variant for SCLC using standard criteria [23]. NSCLC cell lines were derived from adenocarcinoma, squamous cell carcinoma, bronchoalveolar carcinoma (BAC), or large cell undifferentiated NSCLC. The cell lines were all derived from either pleural effusion, lymph node biopsy, or lung tissue.

NCI-N417, NCI-H460, and NCI-H1299 (Table I). These cell lines are documented to be mycoplasma free and were propagated as described [25].

Media

R10 media consisted of RPMI-1640, supplemented with 10% fetal calf serum (Hyclone, Logan, UT) and 1% streptomycin and 1% penicillin. Thymidine content was 6 μM. HITES is a defined media completely deficient of thymidine [26]. It consists of RPMI-1640, and the following additives: hydrocortisone, insulin, transferrin, 17-B-estradiol, sodium selenite, HEPEs buffer, glutamine, and 2% bovine serum albumin.

TABLE II. Seeding Density of Lung Cancer Cell Lines*

Cell line	Cells/ml (R10)	Cells/ml (HITES)
NCI-H23	3.3×10^4	1.7×10^5
NCI-H146	5.6×10^4	1.4×10^5
NCI-H157	5.6×10^4	5.6×10^4
NCI-H187	1.1×10^5	1.1×10^5
NCI-H322	2.8×10^4	3.9×10^4
NCI-N417	3.9×10^4	4.2×10^4
NCI-H460	4.4×10^3	2.2×10^4
NCI-H1299	3.3×10^4	4.4×10^4

*Concentrations of cells from lung cancer cell lines used for MTT assay as performed in R10 media or in HITES media.

Drugs

10-EDAM was supplied in powder form by CIBA-Geigy (Summit, NJ) and dissolved in media. Drug was diluted to a concentration range of 0.1–10,000 nM. DPM was supplied in the intravenous formulation by Boehringer-Ingelheim (Indianapolis, IN). Ampules contained 10 mg DPM/2 ml and also contained 4 mg tartaric acid, 100 mg polyethylene glycol 600, and hydrochloric acid to adjust the pH. Drug was diluted with medium to yield a 0.1–1,000 μM concentration range. MTX was supplied by the NCI-Cancer Therapy Evaluation Program (Bethesda, MD) in powder form and was dissolved in media to yield a concentration range of 1–10,000 nM.

In Vitro Antiproliferative Activity Studies

The MTT semi-automated colorimetric assay [27], which quantitates cell mitochondrial activity based on reduction of a tetrazolium compound by viable cells as determined by a spectrophotometer (540 nm), was used. All assays were performed as previously described [28]. Seeding densities were specific to the individual cell lines used and were based on growth curves in the media used (Table II). There was a mean optical density ± standard deviation of a minimum of six data points per experiment. Each experiment was performed at least in duplicate. The inhibition of clonal growth of human lung cancer NCI-H23 cells by 10-EDAM in R10 and in RPMI 1640 containing 10% dialyzed fetal bovine serum, respectively, was determined according to the method of Benz and Cadman [29].

RESULTS

In Vitro Antiproliferative Activity of 10-EDAM Vs. MTX

Concentrations of 10-EDAM required to cause 50% growth inhibition (IC_{50} values) were lower than MTX IC_{50} in all eight cell lines tested ($P = 0.0078$ by 2-sided sign test). The 10-EDAM IC_{50} values ranged from 4–52 nM, all within the clinically achievable range [5]. The IC_{50} values for MTX in the same human lung cancer cell lines ranged from 35–240 nM. The IC_{50} values

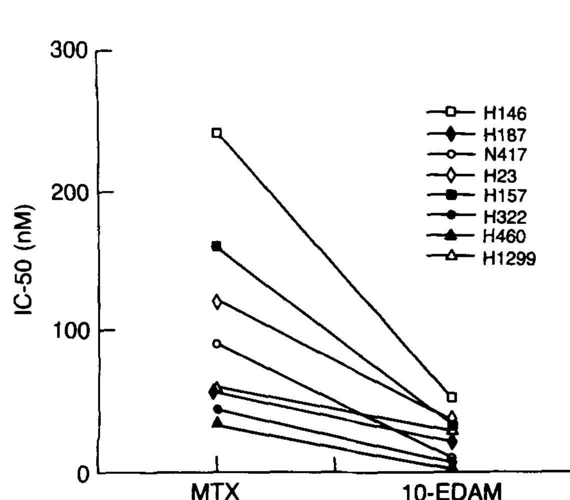


Fig. 2. IC_{50} of MTX and 10-EDAM in lung cancer cell lines. The IC_{50} of MTX and 10-EDAM when tested against eight lung cancer cell lines was derived using the MTT assay. All assays were carried out after 96 h incubation in R10 at 37°C with the concentrations (nM) of drug represented.

for all the cell lines with the two drugs are graphically shown in Figure 2.

The differences in mean IC_{50} values between 10-EDAM and MTX demonstrated an apparent difference in all eight cell lines tested. It was particularly noticeable in four of the cell lines (NCI-H187, NCI-H322, NCI-N417, and NCI-H460), of which two examples are shown in Figure 3. Although the remaining four cell lines showed the same trend, the differences between 10-EDAM and MTX were not as evident. In all of the cell lines tested there were insufficient data points to make direct comparisons of statistical significance for individual cell lines.

In Vitro Antiproliferative Activity of DPM Alone

DPM had minimal antiproliferative activity when tested over a range of 0.1–100 μM for all eight cell lines. Antiproliferative activity, however, was seen in all lines at 500 μM. Survival of the cells approached zero at this concentration of DPM. Figure 4 depicts survival of three representative cell lines over a concentration range of 0–10 μM. As seen, antiproliferative activity was minimal at these drug concentrations. This was the same concentration range used in all subsequent experiments combining 10-EDAM or MTX with DPM.

In Vitro Antiproliferative Activity of 10-EDAM Plus DPM

10-EDAM antiproliferative activity was enhanced by DPM in all eight cell lines tested. The most sensitive cell lines appeared to be NCI-H23, NCI-H146, NCI-H187, NCI-H322, NCI-N417, and NCI-H460. Three representative examples of this additive drug effect are shown in Figure 5 (first three panels). Though marked enhancement was often seen at the lowest DPM concentration used (0.1 μM), maximal relative effect was achieved at 1.0 μM DPM. The effect plateaued with higher concentration. The same trend of enhancement of 10-EDAM antiproliferative activity in combination with DPM was seen in the remaining two cell lines tested (NCI-H157 and NCI-H1299), but was less striking. One of these, NCI-H1299, is shown in Figure 5 (bottom right). By contrast, MTX antiprolifera-

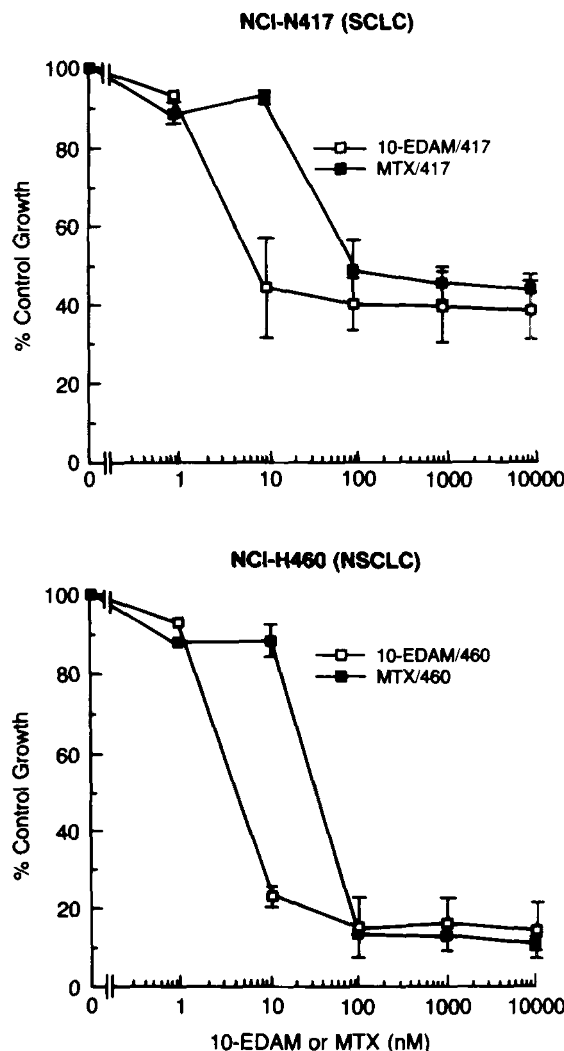


Fig. 3. Antiproliferative activity of 10-EDAM and MTX against human lung cancer cell lines. The antiproliferative activity of 10-EDAM and MTX was tested using the MTT assay at increasing concentrations of drug. Representative cell killing is shown for two cell lines (NCI-H417 and NCI-H460).

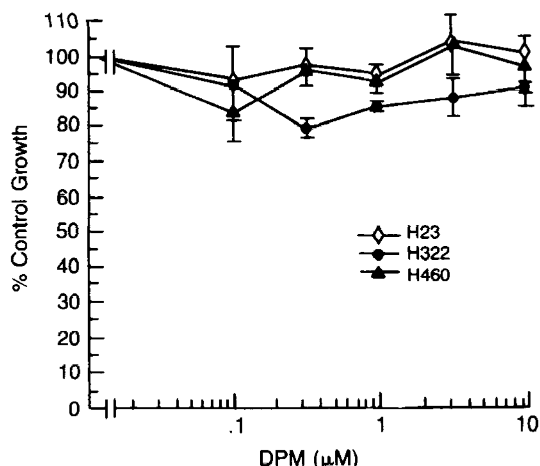


Fig. 4. In vitro antiproliferative activity of DPM. Eight lung cancer cell lines were tested with increasing doses of DPM. The cells were treated at the concentrations shown for 96 h at 37°C in R10. Antiproliferative activity was compared to cells grown in the absence of DPM. Three representative cell lines are depicted in this figure. All cell lines tested were completely killed at 500 μM DPM (data not shown).

tive activity was enhanced by addition of DPM in only two of eight cell lines, NCI-H23 and NCI-H187 (data not shown). In no case did DPM appear to protect the cells from either 10-EDAM or MTX toxicity.

The IC_{50} values for 10-EDAM when given in combination with 1 μM DPM were lower than for 10-EDAM alone in all eight cell lines ($P = 0.0078$ by two sided sign test). The IC_{50} values ranged from 0.66–24 nM when 10-EDAM was given with DPM, compared to a range of 4–52 nM for 10-EDAM alone. A comparison of these values is show in Figure 6.

Folate and Nucleoside Salvage

The cell line NCI-H23 was the most sensitive to the combination of 10-EDAM and DPM. In order to further study the effect of the combination of 10-EDAM and DPM, this cell line was used to study the mechanism of action for DPM enhancement of 10-EDAM antiproliferative activity. A clonogenic assay was used in which NCI-H23 cells were grown in both thymidine replete (R10) and thymidine deficient (HITES) media. When growth media containing serum was used, the IC_{50} value was 47 nM. In the presence of 10 μM DPM, the IC_{50} value was decreased to 15 nM. When these same studies were performed in the presence of dialyzed serum (thymidine free), the IC_{50} values for 10-EDAM alone and in combination with 10 μM DPM were exactly the same, 11 nM. A similar experiment performed with the MTT assay, using media in the absence of thymidine, also showed no enhancement of 10-EDAM antiproliferative activity in the HITES (thymidine depleted) media when DPM was added. However, 10-EDAM alone toxicity was greater in the HITES media than in thymidine replete media (R10) (data not shown).

DISCUSSION

We compared the antiproliferative activity of MTX and 10-EDAM against a range of human lung cancer cell lines. Based on a comparison of the limited clinical data for 10-EDAM [5–7] with the extensive information on MTX in lung cancer [30–33], we predicted 10-EDAM would possess greater antiproliferative activity than MTX in these cell lines. In fact, 10-EDAM did display greater antiproliferative activity than MTX at equimolar concentrations in the eight human lung cancer cell lines tested. Furthermore, 10-EDAM inhibited cell growth in all eight lung cancer cell lines tested at concentrations known to be clinically achievable (0.1–10,000 nM) [5].

Although DPM alone had no significant antiproliferative activity in vitro at concentrations up to 10 μM, the addition of DPM at a clinically achievable concentration of 1 μM decreased the concentration of 10-EDAM required to cause 50% growth inhibition (IC_{50}) in all eight cell lines. A similar effect was seen at concentrations as low as 100 nM DPM, and as high as 10 μM DPM, but maximal relative effect for most lines appeared to occur at 1 μM DPM. In clonogenic assay, enhanced antiproliferative activity with DPM was not observed when the growth media (HITES) deficient in thymidine, folates, or other nucleosides was used. Under these conditions, toxicity of 10-EDAM alone was similar to the toxicity of 10-EDAM plus DPM in replete media (R10). These results suggest that in R10 DPM may enhance 10-EDAM antiproliferative activity by inhibition of thymidine salvage. Further work is necessary to confirm that this is the mechanism of enhanced cell killing by 10-EDAM when combined with DPM.

An important question, given these results, is whether or not DPM could be given clinically at doses that could achieve activity. Recent studies have shown that when DPM is given to patients as a 72 h continuous infusion, total drug levels of 6–13 μM are achieved, although free drug levels ranged from 20–30 nM [15,34,35]. The lowest dose of DPM which enhanced 10-EDAM

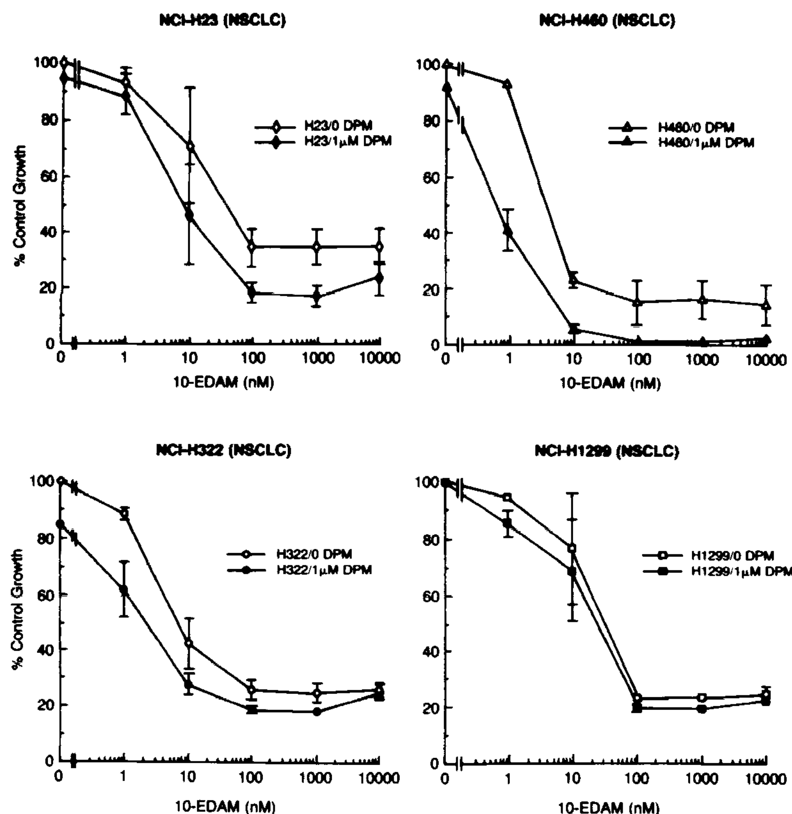


Fig. 5. Effect of combining DPM with 10-EDAM. 10-EDAM was added for 96 h to R10 at the concentrations indicated. DPM was added at a concentration of 1 μM to all cells except where indicated otherwise. Antiproliferative activity was measured using the MTT assay. In six of eight cell lines, marked enhancement was demonstrated by the addition of DPM to 10-EDAM. Three of these cell lines are shown (NCI-H23, NCI-H460, and NCI-H322). Cell line NCI-H1299 demonstrated no significantly enhanced antiproliferative activity.

in vitro that was tested in the present study was 100 nM. This concentration of DPM is close to the range which is felt to be achievable in vivo. This question can best be addressed in a clinical trial.

In summary, 10-EDAM appeared to be a significantly more active agent than MTX in lung cancer cell lines. In addition, the combination of DPM and 10-EDAM had a significantly greater antiproliferative effect than 10-EDAM alone in this group of eight lung cancer cell lines tested, even though DPM had little antiproliferative activity when tested as a single agent. The mechanisms of action of this apparent modulation, as well as possible therapeutic efficacy, are subjects for future study. We believe this combination may have a favorable toxicity profile, is reasonably cost effective, and is potentially associated with antiproliferative activity comparable to existing chemotherapy combinations.

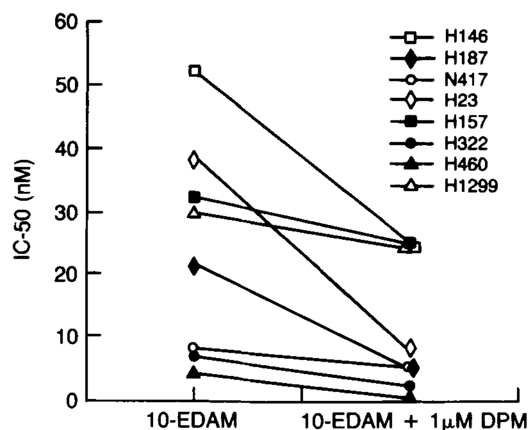


Fig. 6. Comparison of DPM and 10-EDAM antiproliferative activity with 10-EDAM alone. The IC_{50} of the eight lung cancer cell lines for 10-EDAM was determined in the presence of 1 μM DPM in combination with 10-EDAM or with 10-EDAM alone. The data were obtained from growth curves as presented in Figure 5.

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REFERENCES

1. Sirotnak FM, Degraw JI, Moccio DM, Samuels LL, Goutas LJ (1984): New folate analogs of the 10-deaza-aminopterin series. Basis for structural design and biochemical and pharmacologic properties. Cancer Chemother Pharmacol 12:18–25.
2. Sirotnak FM, Degraw JI, Schmid FA, Goutas LJ, Moccio DM (1984): New folate analogs of the 10-deaza-aminopterin series. Further evidence for markedly increased antitumor efficacy compared with methotrexate in ascitic and solid murine tumor models. Cancer Chemother Pharmacol 12:26–30.
3. Schmid FA, Sirotnak FM, Otter GM, DeGraw JI (1985): New folate analogs of the 10-deaza-aminopterin series. Markedly increased activity of the 10-ethyl analog compared to the parent compound and methotrexate against some human tumor xenografts in nude mice. Cancer Treat Rep 69:551–553.
4. Fanucchi MP, Kinahan JJ, Samuels LL, Hancock C, Chou TC, Niedzwiecki D, Farag F, Vidal PM, DeGraw JI, Sternberg SS, Sirotnak FM, Young CW (1987): Toxicity, elimination and metabolism of 10-ethyl-10-deaza-aminopterin in rats and dogs. Cancer Res 47:2334–2339.
5. Kris MG, Kinahan JJ, Gralla RJ, Fanucchi MP, Wertheim MS, O'Connell JP, Marks LD, Williams L, Farag F, Young CW, Sirotnak FM (1988): Phase I trial and clinical pharmacological evaluation of 10-ethyl-10-deazaaminopterin in adult patients with advanced cancer. Cancer Res 48:5573–5579.
6. Shum KY, Kris MG, Gralla RJ, Burke MT, Marks LD, Heelan RT (1988): Phase II study of 10-ethyl-10-deaza-aminopterin in patients with stage III and IV non-small-cell lung cancer. J Clin Oncol 6:446–450.
7. Kris MG, Gralla RJ, Potanovich LM, Marks LD, Heelan RT (1989): Incorporating 10-Ethyl-10-deazaaminopterin (EDAM) into combination therapy with mitomycin and vinblastine for patients with advanced non-small cell lung cancer (NSCLC). Proc Am Soc Clin Oncol 8:884 (Abstr).
8. Wiesenfeld M, Jett JR, Su JQ, Braich TA, Kardinal CG, Mailliard JA, Veeder MH, Morton RF, Michalak JC (1994): Phase II trial of edatrexate in small cell lung cancer. Cancer 73:1189–1193.
9. Schornagel J, Verweij J, de Mulder PH, Cognetti F, Vermoken JB, Cappelaere P, Armand JP, Wildiers J, de Graeff A, Clavel M, Sahmoud T, Kirkpatrick A, Lefebvre JL (1995): Randomized phase III trial of edatrexate versus methotrexate in patients with metastatic and/or recurrent squamous cell carcinoma of the head and neck: A European Organization for Research and Treatment of Cancer Head and Neck Cancer Cooperative Group study. J Clin Oncol 13:1649–1655.
10. Livingston R (1994): Current management of unresectable non-small cell lung cancer. Semin Oncol 21:4–11.
11. Miller V, Rigas JR, Grant SC, Pisters KM, Kris MG (1995): New chemotherapeutic agents for non-small cell lung cancer. Chest 107:306S–311S.
12. Perez E, Hack FM, Fletcher TS, Chou TC (1994): Modulation of intrinsic in vitro resistance to carboplatin by edatrexate in the A549 human nonsmall cell lung cancer cell line. Oncol Res 6:151–156.
13. Fitzgerald GA (1987): Drug therapy dipyridamole. N Engl J Med 316:1247–1256.
14. Grem JL, Fischer PH (1989): Enhancement of 5-fluorouracil's anticancer activity by dipyridamole. Pharmacol Ther 40:349–372.
15. Remick SC, Grem JL, Fischer PH, Tutsch KD, Alberti DB, Nieting LM, Tombes MB, Bruggink J, Willson JK, Trump DL (1990): Phase I trial of 5-fluorouracil and dipyridamole administered by seventy-two hour concurrent continuous infusion. Cancer Res 50:2667–2672.
16. Yang JL, Capizzi R, Contento M (1984): Sequential dipyridamole (DP) retard ARA-C efflux and results in synergistic cytotoxicity. Proc Am Assoc Cancer Res 25: 346 (Abstr).
17. Cabral S, Leis S, Bover L, Nembrot M, Mordoh J (1984): Dipyridamole inhibits reversion by thymidine of methotrexate effect and increases drug uptake in sarcoma 180 cells. Proc Nat Acad Sci (Wash) 81:3200–3203.
18. Nelson JA, Drake S (1984): Potentiation of methotrexate toxicity by dipyridamole. Cancer Res 44:2493–2496.
19. Chan TCK, Howell SB (1985): Mechanism of synergy between N-phosphonacetyl-l-aspartate and dipyridamole in human ovarian carcinoma cell line. Cancer Res 45:3598–3604.
20. Howell SB, Hom DK, Sanga R, Vick JS, Chan TC (1989): Dipyridamole enhancement of etoposide sensitivity. Cancer Res 49:4147–4153.
21. Howell SB, Hom DK, Sanga R, Vick JS, Abramson IS (1989): Comparison of the synergistic potentiation of etoposide, doxorubicin and vinblastine cytotoxicity by dipyridamole. Cancer Res 49:3178–3183.
22. Ramu N, Ramu A (1989): Circumvention of adriamycin resistance by dipyridamole analogues: A structure-activity relationship study. Int J Cancer 43:487–491.
23. Minna JD, Higgins GA, Glatstein EJ (1988): Cancer of the lung. In de Vita V, Hellman S, Rosenberg S (eds): "Principles and Practice of Oncology." Philadelphia: Lippincott, pp 507–598.
24. Allegra CJ (1991): Biochemical modulation: A therapy that has come of therapeutic age. J Clin Oncol 9:1723–1726.
25. Carney DN, Gazdar AF, Bepler G, Guccion JG, Marangos PJ, Moody TW, Zweig MH, Minna JD (1985): Establishment and identification of small cell lung cancer cell lines having classic and variant features. Cancer Res. 45:2913–2923.
26. Simms E, Gazdar AF, Abrams PA, Minna JD (1980): Growth of human small cell (oat cell) carcinoma of the lung in serum-free growth factor-supplemented medium. Cancer Res 40:4356–4361.
27. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB (1987): Evaluation of a tetrazolium-based semi-automated colorimetric assay: Assessment of chemosensitivity testing. Cancer Res 47:936–942.

28. Carmichael J, Mitchell JB, DeGraff WG, Gamson J, Gazdar AF, Johnson BE, Glatstein E, Minna JD (1985): Chemosensitivity testing of human lung cancer cell lines using the MTT assay. Br J Cancer 57:540–547.

29. Benz C, Cadman E (1981): Modulation of 5-fluorouracil metabolism and cytotoxicity by antimetabolite pretreatment in human colorectal adenocarcinoma HCT-8. Cancer Res 41:994–999.

30. Kris MG, Cohen E, Gralla R (1985): An analysis of 134 phase II trials in non-small cell lung cancer. In: Proc of the 4th World Conf on Lung Cancer.

31. Selawry OS (1973): Monochemotherapy of bronchogenic carcinoma with special reference to cell type. Cancer Chemother Rep 4:177–188.

32. Ihde DC, Johnston-Early A, Carney DN, Cohen MH, Bunn PA, Pelsor FR, Minna JD (1982): Lack of efficacy of high-dose methotrexate by 30-hour infusion in patients with progressive small cell carcinoma of the lung. Cancer Treat Rep 66:1223–1225.

33. Hande KR, Oldham RK, Fer MF, Richardson RL, Greco FA (1982): Randomized study of high-dose versus low-dose methotrexate in the treatment of extensive small cell lung cancer. Am J Med 73:413–419.