Treatment Regimens Including the Multitargeted Antifolate LY231514 in Human Tumor Xenografts

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ABSTRACT

The scheduling of antifolate antitumor agents, including the new multitargeted antifolate LY231514 (MTA), with 5-fluorouracil was explored in the human MX-1 breast carcinoma and human H460 and Calu-6 non-small cell lung carcinoma xenografts to assess antitumor activity and toxicity (body weight loss). Administration of the antifolate (methotrexate, MTA, or LY309887) 6 h prior to administration of 5-fluorouracil resulted in additive growth delay of the MX-1 tumor when the antifolate was methotrexate or LY309887 and greater-than-additive tumor growth delay (TGD) when the antifolate was MTA. In the H460 tumor, the most effective regimens were a 14-day course of MTA or LY309887 along with 5-fluorouracil administered on the final 5 days. In addition, the simultaneous combination of MTA administered daily for 5 days for 2 weeks with administration of gemcitabine resulted in greater-than-additive H460 TGD. MTA was additive with fractionated radiation therapy in the H460 tumor when the drug was administered prior to each radiation fraction. MTA administered along with paclitaxel produced greater-than-additive H460 TGD and additive responses along with vinorelbine and carboplatin. In the Calu-6 non-small cell lung carcinoma xenograft, MTA administered in combination with cisplatin or oxaliplatin was highly effective, whereas MTA administered in combination with cyclophosphamide, gemcitabine, or doxorubicin produced additive responses. Administration of MTA along with paclitaxel or doxorubicin resulted in additive MX-1 TGD. Thus, MTA appears to be especially effective in combination therapies including 5-fluorouracil or an antitumor platinum complex.

INTRODUCTION

MTA\(^2\) (N-(4-[2-(2-amino-3,4-dihydro-4-oxo-7H-pyrrolo-[2,3-d]-pyrimidin-5-yl)ethyl]-benzoyl]-l-glutamic acid) was discovered through structure-activity relationship studies based on the novel antipurine antifolate lometrexol (1). MTA contains a pyrrole moiety in the place of the tetrahydropyridine ring of lometrexol, which results in a major shift in activity from the inhibition of de novo purine biosynthesis to predominantly the inhibition of de novo thymidylate biosynthesis (2–4). MTA is an excellent substrate for mammalian folypolyglutamate synthase (5) and, in the polyglutamated form with three or more glutamyl residues, is a potent inhibitor of the enzymes TS, dihydrofolate reductase, and GARFT (2). Mice have relatively high concentrations (about 1 \(\mu M\)) of circulating thymidine. Therefore, evaluation of antitumor compounds with tumors in mice may tend to underpredict both the antitumor activity and toxicity of drugs that inhibit TS as compared with what may be expected in humans (6, 7). To compensate for this potential problem in studying antitumor activity in mice, MTA was tested using a mutant tumor that was thymidine kinase negative, murine lymphoma L5178/Tk/HX, and found to be very active (8). MTA was also found to be an effective antitumor agent against several human tumor xenografts with normal thymidine kinase levels, including the VRC5 colon carcinoma, the GC3 colon carcinoma, the BXPC3 pancreatic carcinoma, the LX-1 non-small cell lung carcinoma, and MX-1 breast carcinoma (1). In several studies, feeding a low-folate diet and then repleting the animals modulated the folate levels in mice by administration of specific doses of folic acid (9). Both the antitumor activity and toxicity of MTA could be modulated in this manner and at certain folate levels, antitumor activity toward specific tumors could be optimized.

An important component in the development of a new anti-cancer drug is an understanding of its potential for inclusion in combination treatment regimens. In recent studies, MTA was tested in combination with cisplatin, methotrexate, 5-fluorouracil, paclitaxel, docetaxel, doxorubicin, LY309887 [GARFT inhibitor (1, 2)], and fractionated radiation therapy in vivo using the EMT-6 mammary carcinoma, the human HCT 116 colon carcinoma, and the human H460 non-small cell lung carcinoma grown as xenografts in nude mice (10). Isothermal methodology was used to determine the additive or synergistic combination regimens. MTA administered with cisplatin, paclitaxel, docetaxel, or fractionated radiation therapy produced additive to greater than additive tumor response by tumor cell survival assay and TGD. Although an additive tumor response was observed when MTA was administered with methotrexate, synergistic tumor responses were seen when MTA was administered with the GARFT inhibitor LY309887 or with the topoisomerase I inhibitor irinotecan. MTA was administered in combination with full doses of each anticancer agent studied, with no evidence of increased toxicity resulting from the combination (10).

In the current studies, MTA was administered alone, in combination with standard chemotherapeutic agents, with a special focus on scheduling with 5-fluorouracil, gemcitabine, and platinum complexes, or with radiation therapy to tumor-
bearing mice to explore the potential interaction of MTA in combination anticancer treatment regimens.

MATERIALS AND METHODS

Drugs

MTA and LY309887 (GARFT inhibitor) and gemcitabine were obtained from Eli Lilly & Co. (2, 11, 12). Cisplatin, carboplatin, cyclophosphamide, methotrexate, 5-fluorouracil, paclitaxel, docetaxel, vinorelbine, and doxorubicin were purchased from Sigma Chemical Co. (St. Louis, MO). Oxaliplatin was purchased from Nescott Fine Chemicals (Santiago, Chile).

Tumors

The Calu-6 human non-small cell lung adenocarcinoma originated from a 61-year old female treated with radiation therapy in 1976 (13). The MX-1 breast carcinoma originated as a poorly differentiated mammary carcinoma in a 29-year old female. Calu-6 cells were purchased from American Type Culture Collection (Manassas, VA). The MX-1 breast carcinoma and H460 human non-small cell lung carcinoma were obtained from the National Cancer Institute-Fredrick Cancer Research Facility, Division of Cancer Treatment Tumor Repository. Each of the tumor cell lines is tumorigenic in nude mice.

Nude mice, male and female, were purchased from Charles River Laboratories (Wilmington, MA) at 5–6 weeks of age. When the animals were 7–8 weeks of age, they were exposed to 4.5 Gy of total body radiation delivered using a GammaCell 40 irradiator (Nordion, Inc., Ottawa, Ontario, Canada). Twenty-four h later, MX-1, Calu-6, or H460 tumor cells (5 × 10⁶) prepared from a brie of several donor tumors were implanted s.c. in a 1:1 mixture of RPMI tissue culture medium and Matrigel (Collaborative Biomedical Products, Inc., Bedford, MA) in a hind leg of the animals. MX-1 tumors grew to 500 mm³ in 34.7 ± 2.9 days, Calu-6 tumors grew to 500 mm³ in 19.0 ± 3.4 days, and H460 tumors grew to 500 mm³ in 14.0 ± 0.8 days.

TGD Experiments

H460 Experiments. Treatments were initiated on day 7 post-tumor cell implantation, when the H460 tumors were approximately 200 mm³ in volume. Animals were treated by with MTA (100 mg/kg) i.p. injection on days 7–13 or days 7–20; with methotrexate (0.8 mg/kg) by i.p. injection on days 7–13 or days 7–20; or with LY309887 (30 mg/kg) by i.p. injection on days 7, 10, and 13 or days 7, 10, 13, 16, and 19 alone or along with 5-fluorouracil (30 mg/kg) i.p., days 7–11 or 16–20 alone or in combinations including an antifolate along with 5-fluorouracil administered on days 7–11 (early) or on days 16–20 (late). Rows, means from two independent experiments; bars, SE.

Fig. 1 Growth delay of the human H460 non-small cell lung carcinoma grown as a xenograft in male nude mice after treatment with MTA (100 mg/kg) i.p., days (d) 7–13 or days 7–20; methotrexate (0.8 mg/kg) i.p., days 7–13 or days 7–20; GARFT inhibitor (LY309887; 30 mg/kg) i.p., days 7, 10, and 13 or days 7, 10, 13, 16, and 19; 5-fluorouracil (5-FU; 30 mg/kg) i.p., days 7–11 or 16–20 alone or in combinations including an antifolate along with 5-fluorouracil administered on days 7–11 (early) or on days 16–20 (late). Rows, means from two independent experiments; bars, SE.
injection on days 7–11 and 14–18 alone or along with oxaliplatin (12.5 mg/kg) by i.p. injection on day 7; oxaliplatin (5 mg/kg) by i.p. injection on days 7 and 14; cisplatin (10 mg/kg) by i.p. injection on day 7; docetaxel (22 mg/kg) by i.v. injection on days 8, 12, and 16; paclitaxel (24 mg/kg) by i.v. injection on days 8, 10, 12, and 15; vinorelbine (10 mg/kg) by i.p. injection on day 8; or carboplatin (50 mg/kg) by i.p. injection on day 8. In the fourth experiment, MTA (100 mg/kg) was administered by i.p. injection on days 7–11 and 14–18 or days 8, 11, 14, and 17 alone or along with fractionated radiation therapy (2, 3, or 4 Gy; GammaCell 40, Nordion Inc., Ottawa, Ontario, Canada) delivered on days 7–11 and 14–18.

**Calu-6 Experiments.** Treatments were initiated on day 7 post-tumor cell implantation, when the Calu-6 tumors were approximately 200 mm³ in volume. Animals were treated with MTA (100 mg/kg) administered by i.p. injection on days 7–11 and days 14–18 alone or along with cisplatin (10 mg/kg) by i.p. injection on day 7; cyclophosphamide (125 mg/kg) by i.p. injection on days 7, 9, and 11; gemcitabine (60 mg/kg) by i.p. injection on days 7, 10, 13, and 16; doxorubicin (1.75 mg/kg) by i.p. injection on days 7–11; oxaliplatin (12.5 mg/kg) by i.p. injection on day 7; or oxaliplatin (5 mg/kg) by i.p. injection on days 7 and 14.

**MX-1 Experiments.** Treatments were initiated on day 7 post-tumor cell implantation, when the MX-1 tumors were approximately 50 mm³ in volume. Animals were treated with MTA (150 mg/kg) administered by i.p. injection on days 7–11, with methotrexate (0.8 mg/kg) administered by i.p. injection on days 7–11, with 5-fluorouracil (30 mg/kg) administered by i.p. injection on days 7–11, or with combinations of these agents administered simultaneously or sequentially, with 6 h between drug injections. In another experiment, MTA (100, 150, or 200 mg/kg) was administered by i.p. injection on days 7–11 alone or along with paclitaxel (24 mg/kg) administered by i.v. injection on days 7, 9, 11, and 13 or along with doxorubicin (1.75 mg/kg) administered by i.p. injection on days 7–11.

The progress of each tumor was measured twice per week until it reached a volume of 4000 mm³. Tumor volumes were calculated as the volume of a hemi-ellipsoid based on tumor diameter measurements made using calipers in two dimensions. TGD was calculated as the time taken by each individual tumor to reach 500 mm³ compared with the time in the untreated controls. Each treatment group included 5 animals, and each experiment was done twice; therefore the number of animals per condition was 10. TGD times (days) are the means ± SE for the treatment group compared with those for the control group (14, 15). Toxicity of the treatment regimens was assessed using change in body weight over the course of the experiments. Body weights were measured twice per week at the same time as tumor diameter measurements.

All in vivo studies were performed in accordance with NIH and American Accreditation Association of Laboratory Animal Care guidelines.
Data Analysis

For determination of additivity, isobolograms were generated for the special case in which the dosage of one agent is held constant. This method allowed determination of additive effect for different levels of the variable agent (16–20). The radiation dose-modifying factor was calculated as the ratio of the radiation dose required to produce 20 days of TGD in the treated and control groups.

Statistical comparisons for the TGD assays were carried out with the Dunnett multiple comparisons test after a significant effect was found by ANOVA (21, 22).

RESULTS

The doses and schedules for the standard chemotherapeutic agents in these studies are standard, widely used regimens for each agent. The doses and schedules used for MTA in these

Table 1  Growth delay of the human H460 non-small cell lung carcinoma grown as a xenograft in male nude mice after treatment with MTA in combination with other cytotoxic anticancer therapies

<table>
<thead>
<tr>
<th>Tumor growth delay (days)</th>
<th>Maximum body weight loss (%)</th>
</tr>
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<tbody>
<tr>
<td>Alone</td>
<td>+MTA</td>
</tr>
<tr>
<td>MTA (100 mg/kg) d7–11; 14–18</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>Cisplatin (10 mg/kg) d7</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>Carboplatin (50 mg/kg) d7</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>Oxaliplatin (12.5 mg/kg) d7</td>
<td>5.2 ± 0.4</td>
</tr>
<tr>
<td>Oxaliplatin (5 mg/kg) d7; 14</td>
<td>7.7 ± 0.7</td>
</tr>
<tr>
<td>Paclitaxel (24 mg/kg) d8, 10, 12, 15</td>
<td>10.8 ± 1.1</td>
</tr>
<tr>
<td>Docetaxel (22 mg/kg) d8, 12, 16</td>
<td>7.9 ± 0.9</td>
</tr>
<tr>
<td>Vinorelbine (10 mg/kg) d7</td>
<td>5.9 ± 0.5</td>
</tr>
<tr>
<td>2 Gy × 10</td>
<td>10.7 ± 0.6</td>
</tr>
<tr>
<td>3 Gy × 10</td>
<td>13.9 ± 1.0</td>
</tr>
<tr>
<td>4 Gy × 10</td>
<td>23.8 ± 1.9</td>
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Fig. 3  Growth delay of the human Calu-6 non-small cell lung carcinoma xenograft grown in female nude mice after treatment with MTA (100 mg/kg) i.p., days (d) 7–11 and 14–18; cisplatin (10 mg/kg) i.p., day 7; cyclophosphamide (CTX; 125 mg/kg) i.p., days 7, 9, and 11; gemcitabine (60 mg/kg) i.p., days 7, 10, 13, and 16; doxorubicin (1.75 mg/kg) i.p., days 7–11; oxaliplatin (12.5 mg/kg) i.p., day 7, or (5 mg/kg) i.p., days 7 and 14, alone or in combinations including MTA. Rows, means of two independent experiments; bars, SE.

Statistical comparisons for the TGD assays were carried out with the Dunnett multiple comparisons test after a significant effect was found by ANOVA (21, 22).

RESULTS

The doses and schedules for the standard chemotherapeutic agents in these studies are standard, widely used regimens for each agent. The doses and schedules used for MTA in these
studies were determined to be optimal for MTA in earlier studies (1, 6–8). The human H460 non-small cell lung carcinoma xenograft is a relatively quickly growing tumor undergoing log-linear growth and reaching a volume of about 4000 mm³ in 36 days post-tumor cell (5 x 10⁶) implantation s.c. in a hind leg of male nude mice. Each of the antifolates (MTA, methotrexate, and 309887) produced a duration-dependent TGD in the H460 lung carcinoma (Fig. 1). 5-Fluorouracil was also an active antitumor agent against the H460 tumor-producing TGDs that were dependent upon the tumor burden at the initiation of treatment. For combination regimens, 5-fluorouracil was administered on days 7–11 along with each antifolate on days 7–13, or 5-fluorouracil was administered on days 16–20 along with each antifolate in the longer regimen on days 7–20. The most effective regimens were the combination of the longer course of MTA treatment along with 5-fluorouracil administration on days 16–20 and the combination of the longer course of 309887 treatment along with 5-fluorouracil administration on days 16–20. The least effective combination regimens were those that included methotrexate. Administration of 5-fluorouracil early in these combination regimens resulted in greater weight loss than administering 5-fluorouracil later.

The antitumor activity of MTA against the H460 tumor is dependent upon dose, duration, and tumor burden. In a daily for 5 days regimen given for 2 weeks, the higher dose of 150 mg/kg per dose was more effective than the lower dose of 100 mg/kg per dose (Fig. 2). Gemcitabine, administered every 3rd day for four doses, was also an active antitumor agent against the H460 tumor. The simultaneous combination of MTA administered on the weekly schedule with administration of gemcitabine resulted in greater-than-additive TGD of the H460 tumor. However, beginning administration of MTA at the completion of the gemcitabine regimen resulted in a less effective therapeutic regimen. Simultaneous administration of MTA and gemcitabine, although a more effective anticancer regimen, was also a more toxic regimen as determined by weight loss, especially with the higher dose of MTA.

Antitumor platinum complexes and antitubulin agents are widely used in the treatment of non-small cell lung carcinoma. Combination regimens with MTA and cisplatin, carboplatin, and oxaliplatin were studied. The antitumor platinum complexes cisplatin and carboplatin were administered once on the first day of the treatment regimen, and the antitumor platinum complex, oxaliplatin, was administered once or once per week for 2 weeks. Although, among the antitumor platinum complexes tested, oxaliplatin produced the greatest TGD in the H460 xenograft, the combination regimen of MTA and cisplatin produced the greatest TGD (P < 0.01; Table 1). Paclitaxel was a more effective single agent than docetaxel or vinorelbine against the H460 tumor. The combination regimen of MTA with simultaneous administration of paclitaxel produced a greater TGD than the combination of MTA with vinorelbine. Each of the combination regimens was more toxic than the single-agent treatments. Cisplatin and oxaliplatin were less toxic in combination with MTA than was carboplatin, and paclitaxel was less toxic in combination with MTA than was docetaxel.

Radiation therapy is also widely used for the treatment of non-small cell lung carcinoma. Radiation therapy was delivered locally to the H460 xenograft tumor-bearing limb in fractions of 2, 3, or 4 Gy administered daily for 5 days for 2 weeks. Radiation therapy produced increasing TGD with increasing dose of radiation (Table 1). The effect of adding treatment with MTA to fractionated radiation therapy was investigated. MTA administration enhanced the TGD produced by radiation therapy and did not add to the toxicity of the radiation therapy.

The Calu-6 non-small cell lung carcinoma was grown as a s.c. xenograft in female nude mice. MTA was an active antitumor agent against the Calu-6 tumor. Combinations of MTA with the antitumor platinum complexes cisplatin and oxaliplatin were tested, with the antitumor platinum complex being administered...
simultaneously at the beginning of the MTA treatment course (Fig. 3). Treatment regimens including MTA with either cisplatin or oxaliplatin were highly effective, producing greater-than-additive TGD of the Calu-6 tumor \( (P < 0.01 \text{ for both cisplatin and oxaliplatin combinations}) \). The antitumor alkylating agent cyclophosphamide was a highly effective antitumor agent against the Calu-6 tumor. The simultaneous combination of MTA treatment and cyclophosphamide was additive against the Calu-6 tumor. The MX-1 human breast carcinoma was grown as a s.c. xenograft in female nude mice. MTA (150 mg/kg) administered by i.p. injection on days 7–11 produced a TGD of 3.0 \( \pm \) 0.3 days in the MX-1 tumor. The simultaneous combination of MTA and 5-fluorouracil produced additive TGD in the MX-1 tumor. When the administration of methotrexate preceded 5-fluorouracil treatment, an additive TGD for the combination was observed. On the other hand, when the administration of MTA preceded 5-fluorouracil treatment, a greater-than-additive TGD resulted \( (P < 0.01) \). The combination treatment regimen of MTA followed by 5-fluorouracil was most effective in maximizing tumor response and produced less weight loss than the other combination regimens; thus, this sequential regimen had a better therapeutic index than the other combination treatments.

Administration of MTA over a dosage range resulted in increasing TGD with increasing dose of MTA in the MX-1 tumor (Fig. 5). Paclitaxel is a very effective single agent against the MX-1 tumor. The simultaneous combination of MTA and paclitaxel resulted in additive TGD of the two antitumor agents over the dosage range of MTA. The MX-1 human breast carcinoma was grown as a s.c. xenograft in female nude mice. MTA (150 mg/kg) administered by i.p. injection on days 7–11 produced a TGD of 3.0 \( \pm \) 0.3 days in the MX-1 tumor. Methotrexate (0.8 mg/kg) administered by i.p. injection on days 7–11 produced a TGD of 2.8 \( \pm \) 0.3 days in the MX-1 tumor. 5-Fluorouracil (30 mg/kg) administered by i.p. injection on days 7–11 produced a TGD of 7.5 \( \pm \) 0.5 days in the MX-1 tumor. The simultaneous combination of methotrexate and 5-fluorouracil resulted in antagonism between the two agents, whereas the simultaneous combination of MTA and 5-fluorouracil produced additive TGD in the MX-1 tumor. When the administration of methotrexate preceded 5-fluorouracil treatment, an additive TGD for the combination was observed. On the other hand, when the administration of MTA preceded 5-fluorouracil treatment, a greater-than-additive TGD resulted \( (P < 0.01) \). The combination treatment regimen of MTA followed by 5-fluorouracil was most effective in maximizing tumor response and produced less weight loss than the other combination regimens; thus, this sequential regimen had a better therapeutic index than the other combination treatments.

Administration of MTA over a dosage range resulted in increasing TGD with increasing dose of MTA in the MX-1 tumor (Fig. 5). Paclitaxel is a very effective single agent against the MX-1 tumor. The simultaneous combination of MTA and paclitaxel resulted in additive TGD of the two antitumor agents over the dosage range of MTA. Doxorubicin was an active antitumor agent against the MX-1 tumor. The simultaneous combination of MTA and doxorubicin resulted in additive TGD of the two agents, with increasing TGD with increasing dose of
DISCUSSION

The folate pathway continues to be a target for anticancer drug development because it is vital to cell survival and appears to be one of the few aspects of cellular metabolism in which there is little or no redundancy. A major challenge in the clinical application of antifolates comes in establishing a therapeutic index for these agents in tumor cells versus sensitive normal tissues such as the bone marrow. One possibility for increasing therapeutic potential is to develop treatment regimens in which the antifolate is used along with another anticancer agent, resulting in a greater effect on the tumor cells than on the normal tissues. The schedule dependence of *in vivo* treatment combinations of methotrexate and 5-fluorouracil were recognized in the 1970s using the murine C3H mammary adenocarcinoma, where administering the methotrexate 6 h prior to 5-fluorouracil resulted in the greatest tumor response (23). These early studies were confirmed and extended to murine colon 38 and several human colon carcinoma xenografts, where it was determined that administration of the antifolate 4–8 h prior to the 5-fluorouracil maximized tumor cell killing without increasing bone marrow toxicity from that of the antifolate alone (24–26). In the human MX-1 breast carcinoma xenograft, the same pattern pertained whether the antifolate was methotrexate or MTA; however, the increased tumor response obtained when MTA preceded 5-fluorouracil was much greater than that obtained with methotrexate. The pharmacological interaction between 5-fluorouracil and an antifolate has been the subject of many studies (27). Two biochemical mechanisms have been put forward to account for the observed additivity. The first relates to increased 5-fluorouracil metabolism to the 5-fluoro-UMP through condensation with phosphoribosyl PP, a metabolite that accumulates following the inhibition of de novo purine biosynthesis. The second relates to enhanced TS inhibition through formation of a ternary complex consisting of TS, 5-fluoro-dUMP and an antifolate. These mechanisms were thought to be the origin of the observed potentiating of 5-fluorouracil by methotrexate (24, 26) and raltitrexed (28, 29), respectively. Given the ability of MTA to inhibit TS, dihydrofolate reductase, and GARFT, this antifolate could possibly modulate the activity of 5-fluorouracil by both of these mechanisms. However, in this regard, direct biochemical analysis has yet to be performed on the tumors treated in this study. Nevertheless, the biological effect of increased tumor response was clear (Fig. 4). Alternative treatment regimens that included an antifolate and 5-fluorouracil were tested in the human H460 non-small cell lung carcinoma; in these regimens, more extended treatments were administered with the antifolate, and 5-fluorouracil was administered early or late in the course of the antifolate treatment. Under these conditions, initiation of the administration of the 5-fluorouracil 10 days into the 14-day regimen resulted in the greatest tumor response with each of the three antifolates (Fig. 1).

A similar scheduling effect was seen with MTA and gemcitabine; that is, a greater antitumor effect was obtained when MTA was given along with gemcitabine than if gemcitabine was administered prior to MTA (Fig. 2).

Gemcitabine has been shown to be a potent radiation sensitizer both *in vitro* and *in vivo* (30–36), whereas in the human H460 non-small cell lung carcinoma xenograft, MTA was additive with radiation (Table 1). MTA has been tested previously in combination with radiation therapy against the human HCT116 colon carcinoma xenograft and found to have an additive effect with radiation in that tumor (10). MTA also had a primarily additive effective in combination with fractionated radiation therapy against the human H460 non-small cell lung carcinoma. The limitation of the application of this finding to clinical trial may be the scheduling of the MTA administration along with the radiation therapy, because MTA was administered prior to each radiation fraction.

Combination of MTA with each of the antitumor platinum complexes (cisplatin, carboplatin, and oxaliplatin) resulted in additive to greater-than-additive tumor response in both the H460 and the Calu-6 non-small cell lung carcinoma xenografts. In these regimens, the antitumor platinum complex was administration as a single dose along with the first dose of MTA (Table 1 and Fig. 3). It also appears that with careful scheduling, MTA can be highly effective when administered along with antitubulin agents, including paclitaxel and docetaxel. Although many of the combination regimens produced greater weight loss in the animals than did the single agents, especially in combinations with 5-fluorouracil, increased antitumor activity appeared more important than the weight loss.

From these preclinical *in vivo* results, it may be concluded that MTA can be combined with other anticancer therapies to therapeutic advantage. Given the prolonged terminal half-life in patients, the scheduling of MTA with other agents could possibly be adjusted to sequential days without loss of the beneficial therapeutic interaction between the agents.

REFERENCES


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