Evidence for Enzymatic Activation and Oxygen Involvement in Cytotoxicity and Antitumor Activity of \( N,N',N'' \)-Triethylenetriphosphoramidate

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ABSTRACT

The cytotoxicity of \( N,N',N'' \)-triethylenetriphosphoramidate (thiotepa) was studied in vitro in the MCF-7 human breast carcinoma cell line and in vivo using the EMT6 mouse mammary carcinoma tumor model, under various conditions of oxygenation and in the presence and absence of Aroclor 1254-induced liver preparations. The cytotoxicity of thiotepa toward exponentially growing MCF-7 cells was markedly dependent on the presence of oxygen during the period of drug exposure, with 3 log greater cell kill at 500 \( \mu \)M thiotepa being observed when the cells were normally oxygenated compared with hypoxic cells. Incubation of thiotepa with an Aroclor 1254-induced rat liver S-9 homogenate, in the presence of a NADPH-regenerating system, resulted in an 8-fold increase in cytotoxicity towards the MCF-7 cells over a wide range of drug concentrations. Thiotepa was shown to be metabolized under these conditions in a NADPH- and \( O_2 \)-dependent reaction that was catalyzed by one or more microsomal cytochrome P-450 enzymes that were present in the S-9 fraction. The thiotepa metabolite triethylene phosphoramidate, which hydrolyzes significantly faster than thiotepa, was significantly less cytotoxic toward the MCF-7 cells than was thiotepa itself, suggesting that it is unlikely to be the S-9 metabolite responsible for the observed increase in drug cytotoxicity. Moreover, triethylene phosphoramidate cytotoxicity was only partially \( O_2 \) dependent and was largely unaffected by incubation in the presence of the S-9 preparation, indicating a mechanism of action distinct from that of thiotepa. Tumor cell survival experiments with the EMT6 mouse mammary carcinoma system revealed that a 3.6-fold increase in thiotepa cytotoxicity was obtained by prior administration of the liver inducer Aroclor 1254 to the tumor-bearing animals, 5 days before drug treatment. Finally, the therapeutic effectiveness of thiotepa was significantly enhanced (3- to 5.8-fold increase in tumor growth delay) when an increase in oxygenation was achieved, by carbogen breathing, in animals given the perfluorochemical emulsion Fluosol-DA. These findings establish that the cytotoxic effects of thiotepa are oxygen dependent and may involve, at least in part, metabolic processes catalyzed by cytochrome P-450 enzymes.

INTRODUCTION

Tepa and thiotepa have been known to have anticancer activity since the early 1950s (1-4). Thiotepa was selected as being superior to tepa for clinical application because of its greater chemical stability (2, 3). Detailing the mechanism of action of thiotepa in vivo, however, has remained difficult (5-9). Both laboratory and clinical studies with thiotepa have suggested that the drug is metabolized to a more active species (5-15). Breau et al. (12) found that thiotepa required in vitro metabolic activation with Aroclor 1254-induced rat liver homogenate to be mutagenic. Recent pharmacokinetic studies have indicated that the alkylating activity present in the urine of patients who were treated with thiotaepa could not be fully accounted for by thiotepa and tepa (13-15).

In our in vitro studies (8), thiotepa was moderately cytotoxic toward EMT6 mouse mammary carcinoma cells, with 1.5 log of cells being killed after exposure to 500 \( \mu \)M thiotepa for 1 h. However, in vivo this drug was extremely cytotoxic, killing 3 logs of EMT6 tumor cells at a dose of 30 mg/kg. It seemed unlikely that metabolism through tepa alone could account for this dichotomy. The present studies were designed to test the hypothesis that further extra- and intratumor metabolism may be involved in the antitumor activity of this drug.

MATERIALS AND METHODS

Drugs. Thiotepa was obtained as a gift from Drs. Robert DeLap and Joseph DeVito, Lederle Laboratories, Inc. (Pearl River, NY). Tepa was obtained as a gift from Dr. Peter Gutierrez, University of Maryland Cancer Center and from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute. Tepa from the National Cancer Institute was further purified by dissolving it in acetone, filtering the insoluble material, drying the solution over anhydrous sodium sulfate, filtering, and evaporating to dryness. The purity of this material was checked by thin layer chromatography and proton nuclear magnetic resonance. Aroclor 1254 was purchased from ACCU Standards (Science Park, NY). The sterile Aroclor 1254-induced S-9 rat liver homogenate preparation was purchased from Microbiological Associates (Bethesda, MD).

Cell Lines. MCF-7 human breast carcinoma cells grow as monolayers in DME supplemented with antibiotics, L-glutamine, and 10% fetal bovine serum. This cell line has a plaing efficiency of 25-40% and a doubling time of 32-36 h in vivo (16). For cloning, cells were suspended by trypsinization, diluted with complete growth medium, and plated onto 60-x 15-mm tissue culture dishes that contained 5 ml of complete growth medium. Colonies grow to a countable size (>50 cells) in about 1 week.

Survival Studies. MCF-7 cells in exponential growth were exposed for 1 or 3 h to concentrations of thiotepa or tepa that ranged from 1 to 500 \( \mu \)M, in DME without serum. Drug exposures of approximately 0.5 \( \times \)\( 10^6 \) MCF-7 cells were carried out in the presence or absence of sterile Aroclor 1254-induced rat liver homogenate (Microbiological Associates) (S-9 fraction, 20.2 mg protein/ml), 250 \( \mu \)g glucose 6-phosphate, 1 mg (300 units) glucose-6-phosphate dehydrogenase, and 120 \( \mu \)M NADP (Sigma Chemical Co., St. Louis, MO) in 5 ml of medium without serum.

For drug exposure under hypoxic conditions, cells were grown in 25-cm² flasks. To produce hypoxia, flasks were fitted with sterile rubber sleeve serum stoppers and exposed to a continuously flowing 95% nitrogen/5% CO₂ humidified atmosphere for 4 h at 37°C prior to drug treatment. These conditions produce a degree of hypoxia sufficient to result in radiobiological resistance (17) (oxygen concentration, 10 ppm or less). Parallel flasks were maintained in a humidified 95% air/5% CO₂ atmosphere. After 4 h, each of the drugs or drug vehicles were added to the flasks by injection through the rubber stopper, without breaking hypoxia. After exposure to each agent for 1 h at 37°C under hypoxia, the cells were washed 3 times with DME and plated for colony formation as described above. Each survival curve was determined in three independent experiments (18).

Tumor Growth. The EMT6 murine mammary carcinoma is an in

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2 To whom requests for reprints should be addressed.
3 The abbreviations used are: tepa, triethylene phosphoramidate; thiotepa, \( N,N',N'' \)-triethylenetriphosphoramidate; DME, Dulbecco's modified Eagle's medium; G6P, glucose-6-phosphate; G6PD, glucose-6-phosphate dehydrogenase.
Concentration range from 5 to 500 μM (Fig. 1). Thiotepa was metabolism of thiotepa to tepa and/or other active metabolites, in parallel without NADPH. Proportion determinations were performed to a standard curve (thiotepa = 0-

A vortex mixer for 30 s. Aliquots of the ethyl acetate diluent (1:1) were analyzed by gas chromatography either directly or after storage at 4°C. Thiotepa quantitation was performed by comparison of peak heights (corrected for the internal standard) to a standard curve (thiotepa = 0-10 nmol) that was generated from assay samples that were incubated in parallel without NADPH. Protein determinations were performed with the Bio-Rad Protein Assay reagent, using bovine serum albumin as standard.

RESULTS

The cytochrome P-450 enzyme system, as well as other enzymes which may be involved in the extra- or intratumor metabolism of thiotepa to tepa and/or other active metabolites, requires oxygen as a cosubstrate. The oxygen dependence of thiotepa toxicity toward MCF-7 cells was therefore examined. Thiotepa killed MCF-7 cells in a log-linear manner over a concentration range from 5 to 500 μM (Fig. 1). Thiotepa was much less cytotoxic toward the MCF-7 cell line under hypoxic conditions than under normally oxygenated conditions (Fig. 1). In fact, at a thiotepa concentration of 500 μM and a 1-h drug exposure time, there was 3 logs less kill of MCF-7 cells by thiotepa under hypoxic conditions than under normally oxygenated conditions.

Experiments were undertaken to determine whether thiotepa was subject to metabolism catalyzed by cytochrome P-450 or other oxygen-dependent enzymes. Incubation of thiotepa with an Aroclor 1254-induced rat liver homogenate (S-9 fraction) led to a time-dependent and protein-dependent loss of intact drug (Fig. 2A). This reaction was dependent on NADPH, with no activity detected using NADH or the oxidized coenzymes NAD and NADP (Fig. 2B). A microsomal fraction prepared from the S-9 liver homogenate retained full enzymatic activity, but a cytosolic fraction did not exhibit any activity (Table 1). Thiotepa metabolism was oxygen dependent and was markedly inhibited by carbon monoxide. Finally, while purified NADPH P-450 reductase was itself inactive in the metabolism of thiotepa (data not shown), antibody to NADPH P-450 reductase was fully inhibitory toward microsomal thiotepa metabolism (Table 1). These findings are all consistent with the proposed involvement of cytochrome P-450 in the thiotepa metabolism catalyzed by the S-9 fraction.

The effect of adding increasing amounts of S-9 fraction on the cytotoxicity of 100 μM thiotepa in the MCF-7 cell line is shown in Fig. 3. Addition to the medium of this S-9 fraction together with a NADPH-regenerating system, increased the cytotoxicity of thiotepa in a dose-dependent manner, with a maximum of about 8-fold increased cell kill being observed at 80 μl (0.32 mg S-9 protein/ml medium that contained 10⁵ cells). In control experiments, the S-9 fraction alone and the NADPH-regenerating system did not exhibit any cytotoxicity toward the MCF-7 cells (Fig. 3).

The enhanced cytotoxicity effected by the S-9 fraction in the presence of the NADPH-regenerating system was observed over a wide range of drug concentrations (5-500 μM), corresponding to a 3-log range of cell kill (Fig. 4). This effect was obtained both when MCF-7 cells were exposed to thiotepa in the presence of rat liver S-9 homogenate and G6P/G6PD/NADP for 1 h and when they were exposed for 3 h, which resulted in a small increase (3-fold) in cytotoxicity. The survival curves in the presence and absence of the S-9 fraction were essentially par-
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A. TIME COURSE

![Graph showing time course of thiotepa metabolism](image)

Fig. 2. Time course and cofactor dependence of S-9-catalyzed thiotepa metabolism. A, Rat liver S-9 fraction was incubated at 37°C with thiotepa (25 μM), in the presence or absence of NADPH (1 mM), for times up to 120 min. Aliquots were removed at the indicated time points and then analyzed for unmetabolized thiotepa by gas chromatography. B, S-9-dependent metabolism of thiotepa was assayed in samples that were incubated for 30 min at 37°C in the presence of the indicated nicotinamide cofactors (1 mM). Shown are mean ± SD (bars) (three independent determinations) for unmetabolized thiotepa recovered at the end of the incubation period, as determined by gas chromatography.

Table 1 Thiotepa metabolism catalyzed by Aroclor 1254-induced rat liver S-9 homogenate and isolated liver microsomes

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-9 homogenate</td>
<td>0.56</td>
</tr>
<tr>
<td>S-9 cytosol</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>S-9 microsomes</td>
<td>1.10</td>
</tr>
</tbody>
</table>

| S-9 reaction in air | 0.31 |
| S-9 reaction in 20% O₂/80% CO | <0.1 |
| S-9 reaction in 20% N₂/80% CO | <0.1 |
| S-9 reaction in air + anti-P-450 reductase* | <0.1 |
| S-9 reaction in air + control IgG | 0.28 |

* Complete reaction mixture (minus NADPH) preincubated with rabbit polyclonal antibody to rat NADPH P-450 reductase (1.5 mg IgG/mg S-9) (29) for 30 min at 22°C. Thiotepa metabolism was then initiated by NADPH addition. This antibody was shown to bind specifically to NADPH P-450 reductase, as judged by Western blot analysis of isolated liver microsomes (30).

parallel, indicating that in the presence of the S-9 fraction the drug is cytotoxic to the same additional aliquot of tumor cells at each dose tested. With 3 h of incubation in the presence of the S-9 fraction, there was a greater increase (10-fold) in tumor cell kill at 100 μM thiotepa than at 500 μM thiotepa, where the increase in tumor cell killing was only 6.5-fold. This suggests that, at very high drug doses, the cytotoxic mechanism becomes saturated so that the additional cytotoxic species formed by the extracellular metabolism of thiotepa was progressively less able to further increase cell killing.

The cytotoxicity toward MCF-7 cells of tepa, a measurable metabolite formed from thiotepa in vivo (13-15), was next examined (Fig. 5). Tepa is chemically much less stable than thiotepa. Therefore, in examining the cytotoxicity of tepa, the level of purity of the tepa used was important. Tepa obtained from the National Cancer Institute contained insoluble materials. This material was further purified in our laboratory, as described under "Materials and Methods." Furthermore, a sample of pure tepa was obtained from Dr. Peter Gutierrez (University of Maryland Cancer Center, Baltimore, MD). Exposure of MCF-7 cells to each of these materials for 1 h resulted in much less cell kill than that observed with thiotepa (Fig. 5). The maximal cell kill produced by tepa at a drug concentration of 500 μM was about 1.5 log, compared to 3.5 log of cell kill with thiotepa at the same concentration and exposure time. Moreover, addition of the rat liver S-9 fraction with G6P/
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Fig. 5. Survival of the MCF-7 human breast carcinoma cell line exposed to various concentrations of tepa obtained from the National Cancer Institute (C), further purified National Cancer Institute material (C), or tepa obtained from Dr. Peter Gutierrez (A), for 1 h, or further purified National Cancer Institute material, for 3 h, in the presence (○) or absence ( ●) of rat liver S-9/G6P/G6PD/NADP. Points, means of three independent experiments; bars, SE.

Fig. 6. Survival of the MCF-7 human breast carcinoma cell line exposed to various concentrations of tepa for 1 h under normally oxygenated (○) or hypoxic (●) conditions. Points, means of three independent experiments, bars, SE.

The perfluorochemical emulsion Fluosol-DA provides a means of increasing oxygenation levels in tumor masses when a high oxygen content atmosphere is breathed in vivo (23–27) (Table 2). The EMT6 mouse mammary carcinoma was used to examine the effect of increased oxygenation on the antitumor activity of thiotahe. The initial half-life of thiotahe in circulation is about 2 h (13–15); therefore, a 2-h carbogen breathing period after drug administration was used. Whether thiotahe was administered at a dose of 7.5 or 10 mg/kg, there was a 1.7- to 1.8-fold increase in tumor growth delay when the animals breathed carbogen for 2 h after drug administration. Fluosol-DA given immediately prior to thiotahe injection produced a small increase in tumor growth delay of 1.2- to 1.3-fold at both drug doses. However, when thiotahe administration was preceded by Fluosol-DA and followed by 2 h of carbogen breathing there was a 5.8-fold increase in tumor growth delay with the lower dose of thiotahe and a 3.0-fold increase in tumor growth delay at the higher dose of thiotahe.

Tumor cell survival curves for EMT6 tumors treated in vivo with single doses of thiotahe and tepa are shown in Fig. 7. Over the dosage range from 10 to 40 mg/kg, both thiotahe and tepa showed log-linear increasing tumor cell kill with increasing drug dose. Although tepa was slightly less cytotoxic than thio-

Table 2 Growth delay of the EMT6 mouse mammary carcinoma produced by a single dose of thiotahe in vivo with increasing drug dose.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Tumor growth delay (days)</th>
<th>7.5 mg/kg thiotahe</th>
<th>10 mg/kg thiotahe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>2.8 ± 0.5</td>
<td>9.0 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Carbogen (2 h)</td>
<td>4.7 ± 0.9</td>
<td>16.3 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>Fluosol-DA/air</td>
<td>3.7 ± 1.2</td>
<td>11.2 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>Fluosol-DA/carbogen (2 h)</td>
<td>16.2 ± 3.1</td>
<td>27.7 ± 2.3</td>
<td></td>
</tr>
</tbody>
</table>

a The EMT6 tumor was grown s.c. in BALB/c mice. Treatment was administered on day 7 after tumor cell implantation, when the tumors were 50–100 mm<sup>3</sup> in volume. Tumor growth delay is the difference in days for treated tumors to reach 500 mm<sup>3</sup>, compared to untreated controls.

b Carbogen (95% oxygen/5% carbon dioxide) breathing was maintained for 2 h after drug administration and then the animals were returned to air.

c Fluosol-DA was administered as a single dose of 12 mg/kg (0.3 ml) i.v. immediately prior to thiotahe administration i.p.

G6PD/NADP had no effect on the cytotoxicity of tepa during a 1-h drug exposure (data not shown). When the drug incubation period was increased to 3 h, tepa was about 1.5 log more toxic over the concentration range examined than it was with the 1-h exposure time. Addition of the rat liver S-9 fraction plus G6P/G6PD/NADP during the 3-h exposure to tepa resulted in a small but not statistically significant increase in tumor cell kill. These findings indicate either that tepa, an oxidized metabolite of thiotahe, is not subject to further metabolism by the S-9 fraction or that such metabolism does not have a significant impact on the cytotoxicity of tepa in the MCF-7 cell line. Also, tepa was less cytotoxic under hypoxic conditions than under normally oxygenated conditions; however, the effect of oxygenation level on the cytotoxicity of tepa was small (Fig. 6) and clearly much less than that seen with the thiotahe (Fig. 1). Interestingly, under hypoxic conditions there was a 3.3-fold greater kill of MCF-7 cells by tepa at a drug concentration of 500 μM for 1 h than there was by thiotahe at the same drug concentration and exposure time.
teta, this difference was not statistically significant. In vivo, therefore, in this tumor system thiota and teta were equally effective cytotoxic agents. In an effort to determine if induction of liver enzymes might lead to greater cytotoxicity from these drugs, tumor-bearing animals were treated with a single dose of Aroclor 1254 5 days prior to treatment with thiota or teta. A small enhancement in thiota cytotoxicity, which increased from 1.7-fold at the 10 mg/kg dose to 3.6-fold at the 40 mg/kg dose, was evident in animals pretreated with Aroclor 1254. On the other hand, with teta the increase in cytotoxicity in the Aroclor 1254-pretreated animals was less than 2-fold over the entire dosage range examined.

**DISCUSSION**

The goal of these studies was to attempt to better understand the cytotoxic action of thiota through an examination of the effect of oxygenation and microsomal enzymes on drug exposure in vitro and in vivo. The impetus for these studies was 2-fold; first, thiota appeared to be a much more cytotoxic agent toward EMT6 mouse mammary tumor cells in vivo than in vitro (8) and, second, more alkylating activity has been found in the plasma of patients who were treated with thiota than could be accounted for by the combined levels of thiota and teta (13–15).

The possibility that hepatic metabolism by cytochrome P-450 or another enzyme system was involved in the conversion of thiota to a more cytotoxic species was first tested biochemically, to determine if an Aroclor 1254-induced rat liver homogenate (S-9) could indeed metabolize the drug, and then by addition of a rat liver S-9 fraction to MCF-7 cell monolayers during exposure to thiota. The S-9 liver fraction catalyzed metabolism of thiota in a NADPH- and oxygen-dependent process that was shown to involve cytochrome P-450. Under these same conditions of metabolism, the S-9 fraction increased the toxicity of thiota to MCF-7 cells up to 10-fold. A similar effect appears to occur in vivo, where Aroclor 1254 pretreatment, under conditions that lead to induction of hepatic P-450 enzymes (28), resulted in a small but significant increase in tumor cell kill by thiota and a smaller but not statistically significant increase in teta cytotoxicity. Consistent with these results are those of Breau et al. (11, 12), who found that teta, at dosages required for antineoplastic activity, induced back-mutation of the histidine operon in strain TA100 of *Salmonella typhimurium* without metabolic activation but that thiota required metabolic activation with an Aroclor 1254-induced rat liver homogenate preparation for TA-100 mutagenic activity. These findings indicate a possible role for one or more cytochrome P-450 enzymes in the extratumor metabolism of thiota.

The thiota metabolite teta was found to be significantly less cytotoxic toward the MCF-7 cells than thiota itself, suggesting that it is unlikely to be the S-9 metabolite responsible for the observed increase in thiota cytotoxicity. Part of this difference may be due to more rapid hydrolysis of teta than thiota in aqueous solution. Moreover, teta cytotoxicity was only partially oxygen dependent and was largely unaffected by incubation in the presence of S-9, indicating a mechanism of action distinct from that of thiota. In contrast, thiota was significantly more toxic toward normally oxygenated than hypoxic cells and was susceptible to S-9-catalyzed increases in cytotoxicity. The differential observed in the present study in the cytotoxicity of thiota toward cells based on their level of oxygenation was one of the largest differences reported for any chemotherapeutic agent (17, 26) and represents the first time that this type of effect has been seen in culture with a classical alkylating agent. The effect of oxygenation level on teta cytotoxicity was much less than that seen with thiota. Under normally oxygenated conditions, teta was about 3-fold less toxic than thiota in vivo, at the 50% survival level in the MCF-7 cell line. Similarly, using P-388 cells in a 72-h growth inhibition assay, Miller et al. (9) found that teta was about 1.9-fold less toxic on a concentration basis, compared to thiota, at the IC50. Teta was more toxic to the MCF-7 parent cell line than thiota under hypoxic conditions. These results are consistent with metabolism of thiota to teta or other active species by the oxygen-requiring cytochrome P-450 or another enzyme system. Alternatively, although the extratumor metabolism of thiota may involve cytochrome P-450, it is possible that the significant cytotoxicity observed in the absence of added S-9 does not involve cytochrome P-450, which is present at very low levels in these cells. In that case, the striking oxygen dependence of thiota cytotoxicity may suggest a role for reduced oxygen species in the action of this drug.

In vivo tumor cell survival data with the EMT6 tumor indicated that, over the dosage range examined, teta was about 1.7-fold less cytotoxic than thiota, although this difference was not statistically significant. In contrast to these findings, Bibby et al. (5) and Phillips et al. (6), studying three murine colon adenocarcinoma cell lines, found that teta was about 1.7-fold more cytotoxic in vitro toward the line most sensitive to thiota in vivo and about 1.3-fold more cytotoxic in vitro toward the lines which were moderately sensitive and resistant toward thiota in vivo. As measured by [3H]thymidine incorporation, Breau et al. (11, 12) found that three human tumor cell lines were equally sensitive to thiota and teta and that one line was more sensitive to teta than to thiota. The variability of thiota- and teta-induced cytotoxicity in vitro may be reflective of differing intracellular levels of enzymes required for thiota activation. In vivo, extratumor and intratumor drug metabolism are factors in the observed antitumor effects. Other tumor physiological factors such as hypoxia and pH, however, also appear to play a role. Thus, teta is more cytotoxic at acidic pH (7), where direct chemical degradation occurs more efficiently, but is much less cytotoxic under hypoxic conditions, possibly because oxygen-dependent metabolism does not occur as efficiently. In order to correct for the relative hypoxic resistance to thiota, we also tested the use of the oxygen-carrying perfluorochemical Fluosol-DA, plus oxygen breathing, in the EMT6 tumor in vivo. This tumor has been shown to contain approximately 15% radiobiologically hypoxic cells (19–22). Fluosol-DA administered prior to thiota, followed by carbogen breathing for 2 h, enhanced the tumor growth delay produced by this drug by about 5-fold at 7.5 mg/kg thiota and about 3-fold at 10 mg/kg thiota. Therefore, the importance of oxygenation level to the cytotoxicity of thiota has been demonstrated both in vitro and in vivo.

The exact nature of the reactive cytotoxic species formed from thiota remains to be elucidated. The studies presented here demonstrate that an enzymatic component in an Aroclor 1254-induced rat liver homogenate can metabolize thiota to a more cytotoxic species and that improving oxygenation level in vivo leads to markedly increased tumor growth delay by thiota. We have shown previously that scheduling of thiota

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* S. Ng and D. J. Waxman, unpublished results.
can affect drug toxicity and tumor cell kill. A knowledge of the components involved in the optimal activation of thiotepa may lead to more effective use of this agent in the clinical treatment of solid tumors.

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Evidence for Enzymatic Activation and Oxygen Involvement in Cytotoxicity and Antitumor Activity of $N,N',N''$ -Triethylenethiophosphoramide


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