In Vivo and In Vitro Enhanced Antitumor Effects by Pentoxifylline in Human Cancer Cells Treated with Thiotepa

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ABSTRACT

Methylxanthines enhance lethality of alkylating agents in human cancer cells, a phenomenon attributed to the prevention of DNA repair. Pentoxifylline is a nontoxic methylxanthine, used clinically for claudication. Using human cancer cells in culture or in a mouse xenograft model, we studied combination treatments with alkylating agents and pentoxifylline or other methylxanthines.

With human bladder cancer cells in culture, cytotoxicity of thiotepa was increased up to 10-fold (P < 0.01) by posttreatment with pentoxifylline, with a major clinical metabolite of pentoxifylline, or with caffeine; the pentoxifylline concentrations required (0.4–1.0 mM) are clinically achievable in the bladder after nontoxic p.o. doses.

With human bladder or breast cancer xenografts in a modified subrenal capsule assay, enhancement of thiotepa was also observed by in vivo posttreatment with pentoxifylline. In contrast, these combinations produced no increased toxicity to normal tissues in these animals, measured by weight, lethality, or histological changes of the normal bladder urothelium.

These results provide evidence for a novel approach to improve the therapeutic index of thiotepa and other alkylators, used for topical therapy of bladder cancer and, possibly, systemic therapy of other malignancies.

INTRODUCTION

MeX enhance the antitumor effects of alkylating agents in human cancer cells in vitro, a phenomenon attributed to the prevention of DNA repair (1). Caffeine is one MeX that has been studied extensively in cell culture and in animal tumor models. Dramatic enhancement of antitumor effects, including improved life span in some rodent tumor models, has been reported in these studies (2–10). In addition, enhancement was observed in rodent tumors which were resistant to standard therapy (2–4), suggesting that such combinations provided a strategy to improve therapy of human cancers with resistance in the clinic (6, 7).

Despite these positive results in various model tumor systems, the clinical use of caffeine is severely limited by its neurological and cardiac toxicities (12, 13). Studies with caffeine in cell culture (1, 14) predict that the 0.4–1 mM concentrations needed to enhance tumor cell lethality exceed the maximally tolerated serum levels by 20-fold and border on the lethal serum level in humans (12, 13, 15).

Our recent studies of another MeX, PENT, indicate that it is nearly equal to caffeine as an enhancing agent in vitro (1). PENT is an oxohexyl-substituted analogue of theobromine, and it has gained wide clinical use for the treatment of intermittent claudication (Trental; Hoechst Pharmaceuticals). The pharmacology of PENT has been well characterized after nontoxic p.o. doses used for claudication therapy (16, 17). These studies indicate that the parent compound and related metabolites are excreted in the urine at levels exceeding 10 mM (16, 17) or 10-fold greater concentrations than those observed to enhance cytotoxicity of alkylating agents in human bladder cancer cells (1).

Since superficial human bladder cancers are often treated with topical instillations of thiotepa or other chemotherapies (18), such MeX concentrations in the bladder provide the opportunity to improve the regional therapeutic effects. Our recent mechanistic studies (1) predict that enhanced cytotoxicity by PENT or other MeX will be selective against cancer versus normal cells in certain clinical settings, based on part on the increased proliferative rate of clinical tumors, e.g., high-grade bladder cancers versus normal bladder urothelium. In addition, these prior experiments (1) and others (8, 14) predict optimal scheduling of PENT-chemotherapy combinations, related to the mechanism of enhancement by modulation of DNA repair in late S-G2 phases of the cell cycle.

The present studies were designed to examine further the potentiation of thiotepa by PENT, and to investigate with systemic and regional bladder toxicity of such combination therapies in an in vivo model. The SRCA allows rapid, economical experimentation with human tumor xenografts (19). Since PENT is rapidly metabolized in vivo (16), this animal model provides a practical system for determination of both efficacy and potential toxicities produced by PENT-chemotherapy treatments after systemic administration in vivo. In contrast to rodent tumor assays, the SRCA allows studies of in vivo therapies targeted to human cancer xenografts, which more closely represent the drug sensitivity, resistance mechanisms, etc., of clinical neoplasia (see Ref. 20 references; Ref. 21).

Our results demonstrate (a) enhancement of thiotepa cytotoxicity by a major clinical metabolite of PENT, studied with human bladder cancer cells in vitro; (b) enhanced in vivo antitumor activity by systemic administration of thiotepa and PENT; and (c) no increased systemic or regional (bladder) toxicity by the thiotepa-PENT drug combination. These preclinical results provide evidence for a novel approach to improve the treatment of superficial bladder cancers, using topical thiotepa in the bladder combined with p.o. administration of pentoxifylline.

MATERIALS AND METHODS

Cells. The T24 cell line, originally established from a human transitional cell carcinoma of the bladder (22), was grown as a monolayer in Dulbecco’s modification of Eagle’s medium supplemented with 10% fetal calf serum, penicillin (100 units/ml), and streptomycin (100 µg/ml) at 37°C in a humidified, 10% CO2 atmosphere.

New cultures were started every 6 weeks from frozen aliquots. The human karyotype was confirmed in our laboratory, and the non-HeLa isoenzyme pattern was confirmed by Dr. Jorgen Fogh (Sloan Kettering Institute for Cancer Research, Rye, NY). Each batch of cells was...
determined to be free of *Mycoplasma* by the method of Schneider et al. (23).

The A1663 cell line, an independent human bladder carcinoma, was grown as a monolayer in Dulbecco's modification of Eagle's medium supplemented with 10% fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml), L-glutamine (2 mM), and N-2-hydroxyethyl piperazine-N-2-ethanesulfonic acid (25 mM) at 37°C, 5% CO₂, and 100% relative humidity.

**Drug Treatment.** Thiotepa (Lederle) was added to cultures from stock solutions dissolved in 0.9% NaCl solution and frozen at ~70°C. Caffeine (Sigma), PENT (Hoechst), and MET I (Hoechst), a major clinical metabolite of PENT (16), were dissolved in 0.9% NaCl solution. All MeX were made fresh as 100 μM stock solutions.

Thiotepa was also injected i.p. (5 or 8 mg/kg) into mice on day 1 (day 5 for MX-1 studies) following tumor implantation. PENT was injected s.c. (257 mg/kg) into mice starting 4 h after thiotepa.

**In Vitro Studies.** Following 1-h exposure by T24 cells to thiotepa, the culture medium was changed, and the cells were trypsinized and plated for cloning efficiency in the presence or absence of MeX. The clonogenic survival of cells was determined by the method described by Fingert et al. (1). To summarize, following exposure to thiotepa during exponential growth, T24 cells were trypsinized and plated for cloning efficiency on standard 60-mm dishes in the presence or absence of MeX. Plates were fixed after 9 days, and colonies greater than 200 cells were identified with crystal violet stain. Percentage of survival was computed as the number of drug-treated tumor colonies relative to controls, so that all data points were corrected for the cloning efficiency of untreated controls.

Clonogenic survival of A1663 cells were performed by using the bilayer soft agar assay described by Alley et al. (24). Analysis of colony formation was performed as below for clinical bladder cancers.

Clinical bladder cancers, taken from surgical resections, were examined by using modifications (24, 25) of the bilayer soft agar technique described by Salmon et al. (26). To summarize, mechanical and enzymatic disaggregation of solid tumors yielded single cells and aggregates of cells (diameter, < 60 μm) suspended in CMRL 1066 medium. One ml aliquots of this cell suspension were applied to agar medium, prepared in the conventional method (26). On day 1, an upper layer consisting of 1 ml standard culture media, 0.3% agarose, and drug was added to the appropriate cultures. Culture dishes were then transferred to a refrigerator (4°C) for 10 min, to room temperature for 10 min, and then to culture incubators (37°C). After 7–21 days, the cultures were examined for tumor colonies with the aid of an inverted stage phase light microscope and scored by a computerized image analyzer, the FAS-II (Omnicon Feature Analysis System, Model II, Bausch & Lomb, Inc.). To improve discrimination of viable tumor colonies versus nonviable clumps or artifacts, the metabolizable vital dye, 2-(p-iodoethyl piperazine-γ-2-ethanesulfonic acid buffer (Sigma). The fibrin clot matrix surrounding the tumor cells. Using a sterilized, triangular metal spatula wetted with cold medium, the pellet was rimmed out of the centrifuge tube into a Petri dish with RPMI 1640 supplemented with penicillin, streptomycin, and 10 μM N-2-hydroxyethyl piperazine-N-2-ethanesulfonic acid buffer (Sigma). The fibrin clot was immediately cut by using a No. 10 scalpel into 1- to 1.4-mm² fragments for implantation.

In addition, passaged MX-1 human breast tumor fragments were used in the SRC assay. To maintain this tumor in continuous passage with stock mice, 1- to 2-mm³ tumor fragments were injected s.c. into the flank of a nude mouse. After 14–20 days, the mouse was sacrificed and the solid tumor was excised and cut into 1- to 1.4-mm³ fragments for direct implantation in the subrenal capsule of immunosuppressed CD-1 mice (27).

The SRC assay followed the technique described by Bogden et al. (28), with several modifications previously described by our laboratory (19, 27). Female CD-1 mice (24–28 g; Charles River Laboratories) were anesthetized with chloral hydrate and swabbed with an ethanol-Zephiran chloride solution. The left kidney was exteriorized through a small incision (approximately 1 cm) in the left flank. A small slit (0.5 cm) was made in the renal capsule with microsurgery scissors or a No. 10 scalpel. The implanted fragment was loaded onto the tip of a 19-gauge trocar and inserted through the slit for deposit under the capsule. Immediately after implantation, the longest and shortest diameters of the implant were measured in *situ* by using a dissecting microscope equipped with an ocular micrometer, calibrated so that 10 ocular micrometer units equaled 1 mm. The kidney was then replaced into the body cavity and the incision was closed with sterile wound clips (Autoclip, Clay Adams).

Mice were randomly assigned to treatment groups after implantation. At the termination of each assay, mice were weighed and sacrificed by cervical dislocation under anesthesia. The left kidney was removed and placed under the dissecting microscope for measurement of final tumor size. Tumor size was computed by the average of the longest and shortest diameters of the xenograft, measured by using the ocular micrometer in a dissecting microscope. Tumor histologies were monitored on day 0 and on the final assay day according to methods previously described (19).

**Immunosuppression.** As previously described (19), we utilized three separate methods for effective, short-term immunosuppression of CD-1 mice. In Method 1, whole-body irradiation of host mice was performed 4–16 h before tumor implantation with a General Electric Maxitron 250 X-ray at 60 rads/min, total dose up to 650 rads. In Method 2, CSA was purchased from Sandoz Pharmaceuticals as a liquid suspension (100 mg/ml) and diluted in oil (Miglyol 812; Dynamit Nobel) prior to injection. CSA injections (80–120 mg/kg) were done s.c. on a daily basis (CSA was omitted on days when chemotherapy was given to avoid drug interactions) starting up to 2 days after tumor implantation. In Method 3, cyclophosphamide (Cytoxan; Mead Johnson) was dissolved in saline immediately before use and injected i.p. (150 mg/kg) 24 h before tumor implantation.

**Toxicity Studies.** Since clinical applications of MeX enhancement may involve both regional (treatment of superficial bladder cancer) and systemic therapy, systemic and regional toxicological changes in mice were evaluated. The systemic toxicity of the MeX-thiotepa drug combination was assessed by monitoring lethality and whole-body weights of CSA-pretreated mice. Through the use of this approach, female CD-1 mice were given injections i.p. of the LD₅₀ or LD₉₀ dose of thiotepa, and mice were then randomized to s.c. injections of saline or PENT (257 mg/kg s.c., given 4 h following thiotepa injection and then daily for 7 treatments). This dose of PENT was chosen because prior studies demonstrated tolerance of daily s.c. injections in CD-1 mice, and this dose approximates (on a molar basis) prior studies with caffeine, used as an enhancing agent in mice (29). Individual weights of the mice were determined at various times following drug treatment, and the average weights of the thiotepa plus PENT group were compared to the average weights of the group receiving thiotepa alone.

Regional toxicity of the drug combination was assessed by comparing histological changes in bladders after treatment with topical thiotepa (5–20 mg/kg in saline, instilled via catheter into bladders of anesthetized mice) or treatment with topical thiotepa plus PENT administered s.c. (257 mg/kg, same schedule as above). After sacrifice of the mice,
the bladders were removed, bisected, and a ring of tissue was removed by using routine histological techniques. For each bladder, specimens were obtained at three tissue levels and stained with hematoxylin and eosin. End points of the studies used microscopic changes, including multiple histological parameters described by Murphy et al. (30) for evaluation of the thiotepa effect in mouse bladders. In particular, nuclear atypia and denudation were evaluated. Histological analyses were performed blindly by using slides that were coded. Groups of mice were studied 48 h and 7 days after exposure to the chemotherapeutic agents in order to assess both the acute damage and the recovery capacity of normal bladder tissues.

RESULTS

In Vitro Studies. After 1-h exposure to thiotepa, 1 mM MeX was added to T24 bladder cancer cells for 24 h. The enhanced lethality of thiotepa by caffeine and PENT in T24 human bladder cancer cells was observed, as previously reported (1). The enhanced lethality of thiotepa by MET 1 was similar to that of the parent compound, PENT (Fig. 1A). The magnitude of the enhancement was slightly greater for caffeine (caffeine > PENT > MET 1); however, all groups treated with MeX showed a statistical (P < 0.01, 2-way analysis of variance) decrease in survival compared to thiotepa alone.

Similar to our results in the T24 cells, the A1663 bladder cancer cells and a fresh clinical bladder cancer specimen exhibited enhanced sensitivity to thiotepa when caffeine was added, studied by clonogenic survival in bilayer agar cultures (Fig. 1, B and C). PENT and MET 1 were not evaluated in these latter bladder tumor cells; these studies were primarily designed (a) to test the reproducibility of MeX enhancement in various bladder cancer cells, studied with an alternative clonogenic assay, and (b) to investigate the MeX concentrations needed to provide such enhancement.

In Vivo Studies. Systemic administration of PENT alone had no effect on tumor growth, but it improved the in vivo antitumor action of thiotepa in our modified SRC model (19). By use of the fibrin clot implantation technique (19), the same passage of human bladder cancer cells was evaluated for enhancement in vivo and in vitro. As shown in Fig. 2, although thiotepa (5 mg/kg) alone showed minimal activity against the T24 bladder cancer cells in vivo, the thiotepa-PENT combination enhanced (P < 0.005) antitumor effect compared to thiotepa alone. Similarly, in vivo enhancement by PENT was observed in MX-1 human breast tumor xenografts (Fig. 3) with a thiotepa dose of 8 mg/kg but not with 5 mg/kg.

Systemic Toxicity. The systemic toxicity of the thiotepa-PENT combination was assessed by monitoring animal body weights and effects of PENT on thiotepa-induced lethality. As shown in Table 1, thiotepa-induced lethality (LD90 and LD50 doses) was not altered by subsequent administration of PENT. The same result was observed in two other independent experiments (data not shown). As shown in Fig. 4, there was no statistical difference between weights of mice given thiotepa alone and mice given thiotepa plus PENT.

In fact, we were surprised in several experiments to observe that mice treated with thiotepa-PENT actually maintained their weight better (P < 0.05; Student’s t test) than the mice treated with thiotepa alone, measured by the nadir of weight loss on day 4. This ameliorative effect of PENT was seen in two other experiments, one examining the effect of PENT on LD90 of thiotepa (Table 1), and another monitoring animal body weights after thiotepa (25 mg/kg) and PENT administration in non-tumor-bearing mice (data not shown).

Regional Toxicity. Histological analysis of thiotepa-induced bladder toxicity in the presence and absence of PENT revealed no increased damage to normal (nonneoplastic) bladder urothelium. Nuclear abnormalities were the most consistent histological finding after thiotepa treatments. These and other changes in the bladder mucosa were not altered by posttreatment with PENT studied 48 h and 7 days after treatment with thiotepa (Fig. 5). Also, PENT did not prevent the recovery capacity of the normal bladder tissues, evidenced by the acute changes in the bladders at 48 h after 5-mg/kg thiotepa treatment, and subsequent return to normal histology by day 7 (Fig. 5). Rep-

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**Fig. 1.** Enhanced lethality by MeX in vitro. **A,** after 1-h exposure to thiotepa, T24 human bladder cancer cells were plated for cloning efficiency and colony survival was determined by the method of Fingert et al. (1). Caffeine (Caf), PENT, and MET 1 (a major clinical metabolite of PENT) produced statistical (P < 0.01, 2-way analysis of variance) decrease in survival compared to thiotepa alone. Bars, SD, B, using the bilayer soft agar method described in "Materials and Methods" and by Alley et al. (24), clonogenic survival of A1663 human bladder cancer cells was decreased by caffeine after treatment with thiotepa. Thiotepa cytotoxicity was increased by caffeine concentrations over 0.4 mM. C, using a clinical bladder cancer specimen, 0.4 mM caffeine was added to the soft agar cultures and the enhancement of thiotepa was observed. SD < 10% in B and C.
Fig. 2. Enhanced in vivo activity of thiotepa (Thio) combined with PENT in T24 human bladder cancer cells using the fibrin clot SRCA (see "Materials and Methods"). Mice were immunosuppressed with cyclophosphamide (150 mg/kg) given i.p. 24 h preimplantation. Thiotepa was given on day 1 (5 mg/kg i.p.) followed by PENT (257 mg/kg) given s.c. 4 and 16 h after thiotepa.

Fig. 3. Enhanced in vivo activity of the combined administration of thiotepa (Thio) (8 mg/kg) and PENT in MX-1 human breast tumor grafts in the SRCA. Mice were immunosuppressed by CSA (80 mg/kg) given s.c. on days 2–4 and 6–9. Thio and PENT were given on day 5 by the schedule and route as in Fig. 2 legend.

Table 1 Effect of PENT on lethality produced by thiotepa. Thiotepa and PENT (257 mg/kg for 2 doses) given by schedule and route as in Fig. 2 legend.

<table>
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<tr>
<th>Thiotepa dose (mg/kg)</th>
<th>Dead/total</th>
<th>+PENT</th>
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<tr>
<td>22</td>
<td>2/10</td>
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<tr>
<td>25</td>
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Fig. 4. Change in body weight of nontumorous mice treated with thiotepa (25 mg/kg) with and without PENT (schedule and route as in Fig. 2 legend). Weight loss on day 4 was lessened (P < 0.05; Student's t test) in PENT-treated mice, and PENT treatment did not inhibit recovery of mouse body weight.

Fig. 5. Histological analysis of bladder toxicity after thiotepa with and without PENT therapy. Anesthetized mice were treated with thiotepa instilled into the bladder for 1 h, then randomized to receive saline or PENT (257 mg/kg) starting 4 h after thiotepa, then daily for 7 doses. On day 2 (left) or day 7 (right) after thiotepa, mice were sacrificed, bladders were fixed in formalin, and histological sections were scored blindly for nuclear abnormalities as described by Murphy et al. (30).

DISCUSSION

Many drugs that are active in cancer therapy damage DNA (31). DNA repair can diminish the cytotoxicity of an agent by several orders of magnitude, as shown by greater lethality in cells genetically defective in a repair process (Xeroderma pigmentosum, ataxia telangiectasia) (32, 33), or by using drugs that inhibit repair (34, 35). Thus, the lethal effectiveness of antineoplastic agents is highly dependent on repair reactions. Repair modulation has been proposed as a strategy to improve therapeutic impact of DNA-damaging drugs, and it is a postulated mechanism for the synergism observed in tumor cell cultures with several agents, including hydroxyurea, 1-β-D-arabinofuranosyl cytosine, nicotinamide, and actinomycin D (36).

The in vitro enhancement of thiotepa lethality to human cancer cells has been observed with several MeX (1, 14). The present study demonstrates the same enhancement phenomenon in vitro with the use of PENT and a major clinical metabolite (MET 1) produced in the systemic circulation after p.o. administration of PENT (16). These results are consistent with studies by our laboratory (1, 14, 37) and by other investigators (8, 38, 39) demonstrating enhancement by substituted methylxanthines and other heterocyclic purine analogues.

In vivo treatment with thiotepa-PENT combinations showed greater antitumor effect against human cancer cells compared to thiotepa treatment alone. In the MX-1 tumor, enhancement by PENT was observed after thiotepa doses of 8 mg/kg but not 5 mg/kg. However, our measurement of tumor size may give a minimal estimate of drug-induced lethality, since the final tumor size includes an unknown amount of dead cells in this in vivo model. In contrast, tetrazolium dye has improved the
discrimination capacity of the in vitro bilayer agar system (24, 25), since it provides a method to eliminate nonviable tumor cell clusters that would artifactually increase colony counts. Similarly, other vital dyes have been used for in vitro tumor cell assays (e.g., thiazolyl blue assay) (40).

Because gross measurements of tumor size in the SRCA can involve both live and dead cells, Levi et al. (41) proposed a modified SRCA that uses microscopic analysis of live versus dead tumor cells to assay drug response. If tumor sizes are measured soon after treatments, little time is allowed for lysis of dead cells within the tumor graft. By use of immunosuppressed mice, we and others (27, 42) have extended the SRC model beyond the 6-day period. This modification allows (a) more time for lysis of dead cells, and (b) greater differences between treated versus controls and, thus, greater capacity to discriminate active treatments (27). Additional studies with histochemical techniques are in progress in our laboratory, to further improve discrimination of the in vivo therapeutic response.

Taken together, the results presented here suggest that PENT acts selectively to decrease the recovery capacity of cancer versus normal cells. These observations are consistent with MeX studies in other in vivo tumor models, demonstrating improved therapeutic response without substantial alterations of toxicity to normal tissues (2, 3, 8, 39). In our present studies, systemic toxicity of the thiotepa versus thiotepa-PENT regimens was evaluated, in part, by monitoring animal body weights after drug administration (43). Alkylating agents such as thiotepa are known to be toxic to the gastrointestinal tract, manifest by the loss of weight, presumably due to nausea and loss of appetite. Thus, we found that PENT posttreatment did not increase the loss of weight in animals treated with thiotepa. In fact, we were surprised to find in three separate experiments that animals treated with thiotepa-PENT fared better than animals treated with thiotepa alone. Also, after an initial weight loss, the animals treated with thiotepa-PENT regained body weight as quickly as animals treated with thiotepa alone. Moreover, our results showed no negative effect of PENT on the LD20 and LD50 produced by thiotepa.

As seen systemically, the thiotepa-PENT combination did not increase regional toxicity to the bladder. There were no significant differences between groups treated with thiotepa alone and groups treated with thiotepa-PENT, monitored by histological examination of changes in bladder urothelium 2 days after drug treatment. In addition, the bladder urothelium of the treated groups was identical 7 days after drug treatment, suggesting that addition of PENT did not impair the recovery capacity of normal cells after thiotepa damage. Our interest in the recovery capacity of normal cells, both in the bladder and in the gastrointestinal tract, arises from the postulated mechanism of MeX enhancement in tumor cells, i.e., modulation of DNA repair after damage by alkylating agents (1). Long-term observation (3 months) of mice treated with these combinations also revealed no untoward effects, including absence of gross tumors. In vivo studies of drug synergism or enhancement, designed to test biochemical modulation at the subcellular level, can be difficult to interpret if the drugs alter each other's pharmacokinetics (44, 45). To avoid such interactions, we routinely began MeX treatment 4 h after thiotepa administration. Using a sensitive gas chromatography technique, Egorin et al. (46) reported a brief half-life of thiotepa in mice (half-life = 9.62 min) and detected little thiotepa in plasma or tissue by 1 h after systemic injection. Thus, although serum or tissue levels of thiotepa were not measured in the present studies, the possibility of direct pharmacological interactions with PENT was unlikely. Moreover, our prior in vitro studies predict that such a 4-h delay would not compromise enhancement by MeX, since the major MeX effect, seen in late S-G2 phases of the cell cycle, commonly occurs with a delay that exceeds 4 h after DNA damage by alkylating agents (1).

The use of standard or high-dose thiotepa, alone or in combination with other chemotherapies, has gained increasing use in clinical trials (47–49). Many of these clinical strategies are based largely on results with L1210, P388, or other animal tumors. However, several investigators have questioned the relevance of such animal tumors to human neoplasia (50–52). Our investigations presented here describe an alternative model to study these and other combination regimens in vivo.

The fibrin clot subrenal capsule assay (19) allows the rapid
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investigation of combination drug regimens, using human tumor histologies that are relevant to future clinical trials. As demonstrated in the present studies, this model provides the additional advantage of allowing the direct comparison of the in vivo and in vitro effects of an experimental therapy, studied in the same passage of human cancer cells. Moreover, this method avoids many problems inherent to passaged human tumor xenografts in nude mice, including altered cell characteristics (or drug sensitivities) after prolonged in vivo passage (53), high expense, and lengthy experimental time (28).

The findings presented here suggest that the systemic administration of PENT after topical thiopeta treatment could improve its therapeutic effects, without risk of increased local or systemic toxicity. The urinary excretion of PENT has been demonstrated in the present studies, this model provides the additional advantage of allowing the direct comparison of the systemic toxicity. The urinary excretion of PENT has been demonstrated in the present studies, and these studies indicate that systemic administration of standard, nontoxic doses achieve urine concentrations of PENT and related metabolites exceeding 10 mM, i.e., 10-fold greater than those required to enhance lethality of alkylating agents (1).

In addition, our results demonstrate PENT enhancement of systemic therapy for human cancer xenografts, and these studies are encouraging in terms of the potential applications to clinical therapy for metastatic disease. However, further studies are required to determine preclinical treatment parameters, which translate into clinical therapies (50). For example, the achievable serum and tissue levels of PENT and its active (enhancing) metabolites remain to be defined in this setting of combination chemotherapy. In this context, it is of interest to note other animal studies, using lower PENT doses than those of our present studies, which suggest that PENT could provide additional advantages to cancer treatment by inhibition of spontaneous metastases (54), and possibly, by improvement in leukocyte function (55) and RBC fluidity (16).

PENT is now used widely for treatment of cladidation, and it produces minimal toxicity in elderly or debilitated patients (16). Our present results and those of other investigators (39, 55) suggest that this drug deserves further study for its potential role in clinical cancer therapy.

ACKNOWLEDGMENTS

We thank Dr. George Prout, Jr., for helpful comments and suggestions.

REFERENCES


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