Detoxification of the Polyamine Analogue \(N^1\)-Ethyl-\(N^{11}\)-[(cycloheptyl)methy]-4,8-diazaundecane (CHENSpm) by Polyamine Oxidase

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ABSTRACT

**Purpose:** Analogues of the naturally occurring polyamines, alkylated on both terminal amines, are being developed as anticancer drugs. Because bisalkylated derivatives of putrescine (1,4-diaminobutane) are potent inhibitors of the flavin adenine dinucleotide-dependent polyamine oxidase (PAO), we asked whether PAO could detoxify synthetic bisalkylated polyamines with chain lengths longer than putrescine.

**Experimental Design:** We investigated the effects of one polyamine analogue in Chinese hamster ovary (CHO) and HCT116 human colon tumor-derived cells, which express dramatically different levels of PAO activity, and in these same cells treated with an inhibitor of PAO.

**Results:** Concentrations of \(N^1\)-ethyl-\(N^{11}\)-[(cycloheptyl)methyl]-4,8-diazaundecane (CHENSpm), ranging from 0.3 to 10 \(\mu\)M, caused growth arrest and reduced cell survival in HCT116 cells but not in CHO cells. This cell line-specific difference in CHENSpm toxicity was not attributable to differences in analogue uptake, because intracellular levels of the drug were similar in CHO and HCT116 cells treated with equivalent concentrations of CHENSpm in the presence of MDL 72,527, a specific inhibitor of PAO. The PAO inhibitor, in combination with CHENSpm, caused a significant increase in intracellular CHENSpm levels and increased growth inhibition and cell damage in CHO cells but not in HCT116 cells. CHO cells, but not HCT116 cells, produced two primary amine-containing metabolites from CHENSpm that were suppressed by MDL 72,527.

**Conclusions:** These data demonstrate that CHENSpm is detoxified in PAO-expressing CHO cells, but not in PAO-deficient HCT116 cells, by a mechanism yielding products containing free primary amine groups and implicate PAO as the detoxification enzyme. Because other studies suggest that PAO may be self-regulated in some tumors, differential expression of PAO may be the basis for selective toxicity of CHENSpm and other \(N\)-substituted polyamine analogues in certain cancers.

INTRODUCTION

Inhibition of polyamine synthesis is detrimental to cell growth and is a strategy for anticancer therapy (1). The bisalkylated polyamine analogues are one of the most effective groups of polyamine-depleting agents (2). These agents consist of spermidine- or spermine-like molecules with conjugated alky groups on both primary amine groups. The toxicity of these molecules is often related to their ability to regulate feedback mechanisms of polyamine metabolic pathways as well as their inability to functionally substitute for naturally occurring polyamines (3).

The mechanisms of cytotoxicity exerted by polyamine analogues are not yet established. The superinduction of SSAT\(^3\) has been investigated as one possible mechanism. SSAT induction is observed in response to some bisalkylated analogues (4), but a correlation between induction of SSAT activity and cell damage is not a general response of all these compounds. Whereas CHENSpm, a bisalkylated tetraamine analogue of spermine, causes only a 3-fold increase in SSAT activity in NCI H157 cells, it induces levels of cell death comparable with that of \(N^1\)-ethyl-\(N^{11}\)-[(cyclopropyl)methyl]-4,8-diazaundecane, which induced SSAT activity several hundred fold (5, 6). SSAT can be highly induced by 1,12-dimethylspermine, without cytotoxicity (7). The bisalkylated polyamine analogues have been reported to induce apoptosis in some cell types (6, 8–10). Compared with the naturally occurring polyamines, this class of polyamine analogues appears to be less efficient inducers of chromatin aggregation (11, 12). By competing with endogenous polyamines for DNA binding sites, synthetic bisalkylated polyamines could render certain areas of the genome susceptible to apoptotic endonucleases.

\(^3\) The abbreviations used are: SSAT, spermidine/spermine \(N^1\)-acyethyltransferase; CHENSpm, \(N^1\)-ethyl-\(N^{11}\)-[(cycloheptyl)methyl]-4,8-diazaundecane; FAD, flavin adenine dinucleotide; PAO, FAD-dependent polyamine oxidase; CHO, Chinese hamster ovary; HPLC, high-performance liquid chromatography.
Bisalkylated putrescine derivatives have been developed as inhibitors of the FAD-dependent PAO (13). These polyamine analogues are nontoxic in several cell culture and animal model systems, except under certain conditions of stress or transformation (14–16). Because of the structural similarity between the PAO-inhibiting bisalkylated putrescine derivatives and the toxic bisalkylated spermidine and spermine derivatives, we wanted to determine whether PAO was a mechanism for detoxifying these latter molecules.

In these studies, we investigated CHENSpm toxicity in CHO cells, which were found to express levels of PAO activity >10-fold higher than other rodent and human cell lines surveyed (17). We compared CHENSpm effects on CHO cells to those in the human colon cancer-derived HCT116 cells, which express low to nondetectable levels of PAO. Finally, we used a specific inhibitor of PAO, MDL 72,527, to modulate putative PAO-dependent processes in these two cell lines.

MATERIALS AND METHODS

Cell Culture and Drug Treatments. CHO K1 cells and human colon adenocarcinoma-derived HCT116 cells were obtained from the American Type Culture Collection (Rockville, MD). CHO cells were grown in Ham’s F-12 medium (Life Technologies, Inc., Grand Island, NY). HCT116 cells were maintained in DMEM (Life Technologies, Inc., with the addition of 1-glutamine and sodium pyruvate (Sigma Chemical Co., St. Louis, MO). All media used for cell culture were supplemented with 10% fetal bovine serum and 1% penicillin (10,000 units/ml)-streptomycin (10,000 mg/ml) solution (Life Technologies, Inc.). Cells were maintained at 37°C in the presence of 5% CO₂ in air. All cells were passaged into new medium every 2–3 days, just before confluence. Drug additions were performed at the time of cell seeding and in the aforementioned media type for each cell line unless otherwise noted. Difluoromethylornithine and the FAD-dependent PAO inhibitor, MDL 72,527, were generously provided by Marion Merrell Dow Co. (Cincinnati, OH). CHENSpm was synthesized as described elsewhere (18).

Cell Number and Viability Determinations. CHO and HCT116 cells were removed from the monolayer by treatment with trypsin (~1500 units/ml; Calbiochem, San Diego, CA)-EDTA (0.7 mM) and counted using a hemocytometer. A sample of the cell suspension was combined in a 1:1 volume ratio with trypsin blue dye (Life Technologies, Inc.), and at least two independently prepared suspensions were counted on a hemocytometer, two counts each. For both cell types, viability was determined by the percentage of cells able to exclude the trypsin blue dye. Cell viability was also assessed using colony-forming assays, as described previously (14). Briefly, after treatments, cells were removed from monolayer cultures, and known cell numbers were plated into Petri dishes in medium and incubated for 2–3 weeks to allow for macrocolony formation. Colonies were stained with crystal violet and counted under a dissecting microscope. Apoptosis was evaluated by light and electron microscopic assessment of cell morphology, as described elsewhere (19).

Electron Microscopy. Transmission electron microscopy was performed as described elsewhere (19). Briefly, cells were washed in PBS and fixed in situ using 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2; EM fixative) for 30 min. The monolayers were then scraped off the culture dish and fixed overnight in EM fixative. Cells were postfixed in 1% osmium tetroxide and dehydrated in a graded series of ethanol and embedded in epoxy resin. One-μm semithin sections were prepared and stained using toluidine blue O counterstained with uranyl acetate and lead acetate and examined by electron microscopy.

Protein Quantitation. Acid-precipitable (0.2 N HClO₄) cellular proteins were dissolved in 0.5 N NaOH, and protein concentrations were measured using the bicinchoninic acid protein assay kit from Pierce (Rockford, IL). Protein concentrations were estimated by comparing the absorbance of unknown samples to that of known BSA standard concentrations in the same assay.

Polyamine Oxidase Assays. Polyamine oxidase activity was determined by the method of Carper et al. (17). Cells were seeded at 2 × 10⁵ cells/150-mm dish (Costar) and collected at 24-h intervals. Cells were washed twice in ice-cold PBS (140 mM NaCl, 2 mM KCl, 8.1 mM Na₂HPO₄, and 0.9 mM K₂HPO₄) and counted with an electronic particle counter (Coulter Electronics, Inc., Hialeah, FL). Cell pellets were resuspended in 50 mM glycine/NaOH buffer (pH 9.5) and stored at −80°C before assay. Samples of the thawed and clarified supernatant were adjusted to a final concentration of 25 μM MDL 72,527 and incubated on ice for 30 min. These control samples without the inhibitor were then incubated at 30°C in a total volume of 500 μl of assay mixture containing 50 mM glycine/NaOH (pH 9.5), 0.82 mM aminoantipyrine, 10.6 mM phenol, 5 mM N⁴-acetylspermine, 25 mM aminotriazole, and 1 unit of horseradish peroxidase. HCT116 cell lysates were incubated for 2 h, whereas CHO cell lysates were incubated for 30 min/assay. H₂O₂ formation was estimated by the peroxidase-coupled formation of dye product. This was quantified spectrophotometrically by A₅₃₀. One unit of enzyme activity is defined as 1 pmol of N⁴-acetylspermine oxidized/min of reaction incubation.

Polyamine Analysis. Cells were harvested and sonicated in the presence of 0.1 N HCl. To precipitate cellular proteins, the cell lysate was adjusted to 0.2 N HClO₄ and incubated overnight at 4°C. The acid-soluble fraction was obtained by centrifugation at 15,000 × g for 10 min at 4°C. CHENSpm and naturally occurring polyamine levels were measured by HPLC, using a precolumn derivatization method described by Wu et al. (18) or a derivatization method described by Carper et al. (17).

RESULTS

PAO Activity in CHO and HCT116 Cells. PAO activity was measured in both cell lines over a 4-day interval after subculture (Fig. 1). In CHO cells, PAO activity increased and then decreased as a function of time after subculture, with values ranging from 100 to 500 units/mg protein. In HCT116 cells, maximal PAO activity was <10 units/mg protein during the 4-day assessment interval.

Toxicity of CHENSpm Treatment in HCT116 and CHO Cells. When HCT116 cells were treated with CHENSpm, cell growth was inhibited in a concentration-dependent manner (Fig. 2A). Concentrations as low as 1 μM caused a reproducible
show characteristic features of apoptosis, as described by Kerr appearance of apoptotic cells. Electron microscopy failed to treated with the PAO inhibitor) was not associated with the treatment in HCT116 cells and in CHO cells (in this case, when CHO cells were treated with the PAO inhibitor MDL 72,527 resulted in CHENSpm-induced cytotoxicity in this cell line. CHENSpm toxicity was verified in these cells by assessment of cell survival, measured by colony-forming assays. As seen in Fig. 3B, treatment of HCT116 cells with CHENSpm concentrations ranging from 1 to 10 µM reduced cell survival in a dose-dependent manner. Both methods estimated equivalent levels of cytotoxicity (~50% cell killing) for a 24-h exposure to 10 µM CHENSpm.

Neither cell growth (Fig. 2B) nor viability (Fig. 3A) was affected in CHO cells treated with 10 µM CHENSpm. However, when CHO cells were treated with the PAO inhibitor MDL 72,527 along with CHENSpm, cell viability decreased as a function of time (Fig. 3A). Inhibition of PAO with MDL 72,527 resulted in CHENSpm concentration-dependent loss of cell viability in CHO cells, measured by vital dye exclusion (Fig. 3C). Thus, CHENSpm induced concentration-dependent toxic effects in PAO-deficient HCT116 cells and in CHO cells treated with PAO inhibitor.

The decrease in cell viability resulting from CHENSpm treatment in HCT116 cells and in CHO cells (in this case, when treated with the PAO inhibitor) was not associated with the appearance of apoptotic cells. Electron microscopy failed to show characteristic features of apoptosis, as described by Kerr et al. (20), in CHENSpm-treated HCT116 (Fig. 4) or CHO cells (Fig. 5). Treatment with 25 µM MDL 72,527 was nontoxic to CHO cells, as measured by vital dye exclusion, colony formation (14), or electron microscopy (Fig. 5E). However, the PAO inhibitor increased CHENSpm-induced damage in this cell line, as seen qualitatively in Fig. 5, B, C, and D compared with F, G, and H, and quantitatively (membrane damage measured by vital dye exclusion) in Fig. 3C. In these latter two experiments, CHENSpm concentrations were increased to 50 µM to detect possible apoptosis. Features of necrosis were evident in CHENSpm-treated cultures, and this type of cell death was accentuated by MDL 72,527 (Fig. 5), but no classic apoptosis was observed in either of the two cell lines investigated in this study under the conditions reported.

Polyamines and CHENSpm in Treated Cell Lysates.

Our HPLC method of polyamine detection, using postcolumn derivatization of free primary amines, detects a number of mono-, di-, tri-, and tetra-amines (Fig. 6A). CHO cells contain several major species of amines with free terminal amine groups (Fig. 6B). These include several unidentified monoamines, putrescine, spermidine, and spermine. HPLC analysis of acid extracts from CHENSpm-treated CHO cells revealed two new species (Fig. 6C) not found in untreated cells. The species marked X and Y in Fig. 6C (CHENSpm) do not correspond with any peaks seen in Fig. 6B (no CHENSpm). These species were suppressed when CHENSpm-treated CHO cells were also treated with MDL 72,527 [compare Fig. 6C (without MDL 72,527) and Fig. 6D (cells treated with MDL 72,527)]. Because CHENSpm is a bis(alkylated) molecule and the HPLC methods used rely on a free amino terminus for reaction with a fluorescent conjugate, CHENSpm itself cannot be detected in this...
However, these data suggested that PAO was metabolizing the analogue into a new form, or forms, containing a free primary amine group.

This suggestion was corroborated by direct measures of polyamine contents. As shown in Table 1, the analogue was detectable in extracts of HCT116 cells treated with 10 μM CHENSpm. In contrast, CHENSpm was not detectable in CHO cells treated with 10 μM CHENSpm alone. However, treatment with the PAO inhibitor MDL 72,527 dramatically increased the content of CHENSpm in CHO cells (3.4 nmol/mg protein) to levels similar to those found in HCT116 cells (~6 nmol/mg protein) independent of treatment with MDL 72,527. It should be noted that the results shown in Fig. 6 are HPLC chromatograms not normalized to protein content. When these values are normalized, it can be seen that CHENSpm had only a modest effect on the steady-state polyamine contents in CHO cells (Table 1) but did reduce the levels of putrescine and spermidine by up to 45% in HCT116 cells.

**DISCUSSION**

Cytotoxicity resulting from polyamine and polyamine analogue accumulation has been reported in a number of cell lines (5, 8–10, 16, 21). The present study suggests that the FAD-dependent PAO protects mammalian cells from the cytotoxic effects of the bisalkylated polyamine analogue CHENSpm. PAO may also act, along with other mechanisms regulating intracellular polyamine pool sizes, to prevent toxicity associated with accumulation of high levels of endogenous polyamines. Loss of these regulatory mechanisms, resulting in high levels of intracellular polyamines, is sufficient to induce apoptosis (21). PAO has the capability, in concert with SSAT, to convert the tetraamine spermine into diamines, which are substrates for a diamine exporter (22). Thus, PAO can convert longer chain and more positively charged amines to shorter chain and less positively charged molecules and potentially facilitates their removal from cells via specific export mechanisms.

Four lines of evidence indicate that CHENSpm is metabolized by PAO in mammalian cells: (a) CHO cells, which express high levels of PAO, are refractory to the toxic effects of CHENSpm, compared with HCT116 cells, that express low to nondetectable levels of this enzyme; (b) a specific PAO inhibitor enhances CHENSpm toxicity in CHO cells but not in HCT116 cells; (c) authentic CHENSpm is undetectable, by HPLC methods, in CHO cells but is readily observed when these cells are treated with the...
PAO inhibitor. CHENSpm is detectable, at the same concentrations, in HCT116 cells independent of treatment with the PAO inhibitor; and (d) two CHENSpm-dependent amines, each containing at least one free primary amine, are detectable in acid extracts of CHO cells, but not HCT116 cells, treated with this polyamine analogue. These novel amines are suppressed in CHO cells treated with the PAO inhibitor, suggesting that these amines represent products of a PAO-mediated oxidation reaction. MDL 72,527 is a potent and highly specific inhibitor of the FAD-dependent PAO and does not inhibit related oxidases such as the monoamine or diamine oxidases (23).

Because our HPLC method is ion paired, the retention times of the putative CHENSpm metabolites seen in HPLC chromatograms indicate that one molecule contains three amino groups, whereas the second metabolite contains four amino groups. This suggests that one cleavage is occurring to liberate cycloheptyl-aminopropionaldehyde and N-ethyl-spermidine. Another potential product, which matches a peak seen in the chromatograms, is the release of cycloheptyl-aldehyde. This would produce a tetraamine that is larger than spermine and may correspond to the peak that elutes after spermine in the CHENSpm-treated HPLC samples. Confirmation of these spe-

lysomes with a swollen or vacuolated appearance. Cells treated with 50 μM CHENSpm alone (D) showed an increase in the number of small- to medium-sized (arrow) secondary lysosomes. Cells treated with the combination of 50 μM CHENSpm and MDL 72,527 (H) showed a marked increase in the number of secondary lysosomes (arrow). A uranyl acetate, lead citrate counterstain was used.

**Fig. 5** Electron micrographs of CHO cells treated with increasing concentrations of CHENSpm in the presence and absence of the PAO inhibitor MDL 72,527. Cells were treated for 4 days with 0 (A and E), 10 (B and F), 25 (C and G), or 50 (D and H) μM CHENSpm. Cultures were simultaneously maintained in the absence (A, B, C, and D) or presence (E, F, G, and H) of the PAO inhibitor MDL 72,527 (25 μM). Cells treated with MDL 72,527 alone (E) or 10 μM CHENSpm alone (B) were indistinguishable from untreated control cells (A). Cells treated with the combination of 10 μM CHENSpm and MDL 72,527 (F) revealed a marked increase in the number of secondary lysosomes with a swollen or vacuolated appearance (arrow). Cells treated with 25 μM CHENSpm alone (C) showed an increase in secondary lysosomes, most of which did not have a swollen appearance. Cells treated with the combination of 25 μM CHENSpm and MDL 72,527 (G) showed a marked increase in the number of small (arrow) and large secondary lysosomes with a swollen or vacuolated appearance. Cells treated with 50 μM CHENSpm alone (D) showed an increase in the number of small- to medium-sized (arrow) secondary lysosomes. Cells treated with the combination of 50 μM CHENSpm and MDL 72,527 (H) showed a marked increase in the number of secondary lysosomes (arrow). A uranyl acetate, lead citrate counterstain was used.

**Fig. 6** HPLC analysis of CHO cells treated with CHENSpm and/or MDL 72,527. HPLC chromatograms represent standard preparations of authentic polyamines (A), acid extracts from untreated CHO cells (B), CHO cells treated with 10 μM CHENSpm (C), or CHO cells treated with 10 μM CHENSpm and 25 μM MDL 72,527 (D). Cells were treated for 4 days and then harvested for polyamine analysis. Extracts and HPLC procedures were performed as described in “Materials and Methods.” Put, putrescine; Dah, diaminoheptane; Spd, spermidine; Spm, spermine.
Detoxification of CHENSpm by Polyamine Oxidase

The major finding of this study is that the toxicity of CHENSpm, and potentially other bis(alkylated) polyamine analogues, is modulated by the FAD-dependent PAO, which is expressed in a cell type-specific manner. As discussed in the previous paragraph, CHENSpm-induced toxicity occurs via an apoptosis-independent mechanism in CHO and HCT116 cells. CHENSpm toxicity may occur via a lysosome-related mechanism, because our electron microscopy studies show dramatic morphological changes associated with this organelle in CHENSpm-treated cells (Figs. 4 and 5). Recently, Dai et al. (16) demonstrated that MDL 72,527, the PAO inhibitor used in these studies, induced apoptosis in a transformed rodent hematopoietic cell line via a lysosomotropic mechanism. As seen in Fig. 5E, no detectable morphological changes are detected in CHO cells treated with 25 μM MDL 72,527 continuously for 4 days. We did not observe morphological changes or decreases in viability, measured by either vital dye exclusion or colony-forming assays, in either of these cell lines when treated at this concentration (data not shown). This concentration of MDL 72,527 does inhibit PAO activity in CHO cells, as shown previously (14, 17). Thus, the CHENSpm-induced lysosomal alterations, which are accentuated by the PAO inhibitor, are not strictly a direct consequence of the toxicity of the PAO inhibitor. Studies are in progress to further define the role of lysosomes in CHENSpm-induced toxicity.

The role of PAO in detoxification of CHENSpm may have useful implications for this agent, and other bisalkylated polyamine analogues, in cancer therapy. Inhibition of PAO has been shown to prevent the catabolism of the polyamine analogue N,N'-bis[3-(ethylamino)propyl]-1,7-heptane diamine) and also potentiate the antitumor activity of this analogue in a murine leukemia model (26). Our data from HCT116 cells suggest that PAO levels may be a pivotal factor in the efficacy of polyamine analogues against human colonic neoplasias. Linsalata et al. (27) have reported that PAO activity is self-regulated in neoplastic colonic tissue, compared with adjacent normal colonic mucosa. This finding is consistent with previous studies from our group indicating that PAO activity is low to nondetectable in species will require additional studies (e.g., gas chromatography/mass spectroscopy methods), which are in progress.

These data indicate that the FAD-dependent PAO has a protective effect on cells treated with the bisalkylated polyamine analogue CHENSpm. CHO cells contained up to 500 times more PAO activity than do HCT116 cells and were unaffected by the presence of 10 μM CHENSpm. HCT116 cells treated in a similar manner were severely growth inhibited. When CHO cells were treated with CHENSpm and PAO inhibitor, growth was suppressed in a CHENSpm concentration-dependent manner. Along with evidence for metabolism of CHENSpm by PAO to species containing free primary amine groups, as discussed above, these results are consistent with previous findings that indicate that bisalkylated polyamine analogues are more toxic than monoaalkylated derivatives (2). It should be noted, however, that the metabolism of the bisalkyl polyamine analogue M27695 by PAO yields a derivative that is more toxic than its parent compound (24). Taken together, these data emphasize that PAO plays a critical role in the modulation of polyamine analogue toxicity, an important consideration when these analogues are to be used as chemotherapeutic agents.

We have not determined whether other N-substituted polyamine analogues are metabolized by PAO. Addressing this question in the future will be facilitated by the recent cloning of the human PAO gene (25).

CHENSpm did not appear to induce apoptosis in either HCT116 or CHO cells, and this result was not changed by treatment with the PAO inhibitor. Electron microscopy failed to detect the morphological changes (e.g., chromatin condensation and margination, nuclear segregation, nuclear fragmentation, formation of apoptotic bodies, and others), which are hallmarks of apoptosis (20), in CHENSpm-treated HCT116 cells or CHO cells treated with the PAO inhibitor. Previous studies have reported that CHENSpm can induce apoptosis. This result was most readily apparent, as assessed by morphological criteria, in non-small cell lung cancer cells treated with this agent (6). CHENSpm has also been shown to induce features of apoptosis, including DNA fragmentation, in cell lines derived from epithelial cancers of the breast and prostate (8, 10), but these reports do not confirm CHENSpm induction of morphological changes characteristic of apoptosis. Thus, it is possible that CHENSpm-induced toxicity may be expressed in the form of apoptosis in some cell types (e.g., human non-small cell lung cancer) but not in others (e.g., CHO and HCT116 cells as shown here). Even in non-small cell lung cancer cells, CHENSpm appears to induce toxicity by a mechanism(s) independent of caspase activation (6).

The differences between PAO expression in CHO and HCT116 cells described here are not attributable to species-specific differences. We have reported previously that PAO expression is cell type specific in both rodent and human cells (17).

Studies are in progress to further define the role of lysosomes in CHENSpm-induced toxicity.

## Table 1

<table>
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<th>Cell line</th>
<th>Treatments</th>
<th>CHENSpm</th>
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* Cells were treated for 4 days with 10 μM CHENSpm and 25 μM MDL 72,527 as indicated.

Values shown represent duplicate determinations from a single representative experiment, which has been replicated. Variability between duplicate determinations was <2% of the mean value for samples shown here.

ND, not detectable; limit of detection, 0.05 nmol/mg protein.
human tumor-derived cells (17). A human PAO has been cloned recently and shown to be induced by bis(ethyl)norspermine in human lung tumor cells (25). The data shown here support the conclusion that the sensitivity of cells to polyamine analogues, such as CHENSpm, can be influenced by inducible levels of polyamine catabolic enzymes including PAO.

Intracellular polyamine pools in all cell types are regulated in a complex manner by opposing synthesis and catabolism and uptake and efflux mechanisms (28). Up-regulation of polyamine synthesis is a strategy used by cancer cells in some models, including one genetic model of intestinal cancer (29). SSAT works in concert with PAO in polyamine catabolism. Consequently, both up-regulation of synthesis and uptake and down-regulation of polyamine catabolic and export mechanisms may be strategies used by some cancers to maintain high levels of polyamines in support of the neoplastic phenotype. Thus, CHENSpm, and possibly other bisalkylated polyamine analogues, may exhibit selective cytotoxicity against neoplastic, compared with normal, colonic mucosal tissues, attributable to a failure to detoxify these agents in the neoplastic tissues. Further assessment of PAO expression in neoplastic colonic and other tissues will be important to substantiate, or disprove, this possibility.

REFERENCES

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