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Review

Polyamine–DNA Interactions: Possible Site of New Cancer Chemotherapeutic Intervention

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The effects of polyamines on the structure of nucleic acids in cell-free systems and in cell culture systems are reviewed. Evidence suggests that polyamine depletion inhibits cell growth and may cause conformational changes in DNA. These effects may be exploited to cause changes in the action of drugs and may be used to advantage in combination treatment protocols. A discussion of theoretical models of the interactions, physicochemical evidence for conformational changes, and the effects of anticancer drugs in cells depleted of polyamines is presented.

KEY WORDS: polyamines; nucleic acids; nucleic acid conformation; molecular modeling; cancer chemotherapy.

INTRODUCTION: METABOLISM AND STRUCTURE OF POLYAMINES

The polyamines are an intriguing class of simple aliphatic polycations (Fig. 1). The relationship of polyamines to the structure and function of both prokaryotic and eukaryotic cells—especially the relation to growth—has been reviewed recently (1,2). Putrescine, spermidine, and spermine, the natural polyamines most prevalent in mammalian cells, are the product of an exquisitely regulated biosynthetic pathway (Fig. 2) in which ornithine is decarboxylated to putrescine by the enzyme ornithine decarboxylase (ODC) (for review, see Ref. 3). Putrescine is converted to spermidine by the addition of an aminopropyl group donated by decarboxylated *S*-adenosylmethionine (decarboxy AdoMet); addition is catalyzed by the aminopropyl transferase spermidine synthase. Spermidine in turn is converted to spermine by the addition of a second propylamine group from the same donor, with the transfer catalyzed by spermine synthase. *S*-Adenosylmethione is decarboxylated by the enzyme *S*-adenosylmethionine decarboxylase (AdoMet DC). The enzymes ODC and AdoMet DC have very short half-lives and turn over very rapidly, which teleologically suggests their participation in regulating processes. The complete biosynthetic pathway, as it is currently understood, is considerably more complex than the general pathway just discussed. For instance, the pathway is reversible, compounds less polar than the parent molecule can be formed by acetylation (this interconversion is important in considering intracellular distribution), and for ODC particularly, a number of known and possible regulatory mechanisms such as antizyme activity and phosphorylation have been described (for a review, see Ref. 3). For the purposes of this review, it is sufficient to note that the regulation of the synthesis and interconversion of these simple compounds is an exceptionally rich and complex process.

Various activities of the polyamines are known and significant progress is being made to relate these activities to the molecular mechanisms that control a variety of cellular functions (4). Intracellularly, polyamines are protonated and may participate with inorganic cations in a variety of processes that maintain normal cellular structure and function. Specific cationic functions of the polyamines that cannot be duplicated by other cations at physiologic concentrations are the subject of intense investigation. The specificity of interaction may be related to the fact that the positive charges of polyamines are distributed over the length of the molecule and, unlike inorganic cations, are not point-localized charges. The regulation of polyamine biosynthesis and their metabolic interconversion add a dimension to the intracellular functions of these compounds that is not possible for inorganic cations.

POLYAMINE BINDING TO NUCLEIC ACIDS

The association of polyamines with nucleic acids (4,5) has been of particular interest to our laboratory. Early studies showed that the polyamines are associated with nucleic acids isolated from viral, bacterial, and mammalian cells. Polyamines cause the precipitation of nucleic acids, increase their melting temperature, and protect them against enzymatic degradation, X-irradiation, and mechanical shear in cell-free systems. Polyamines also promote the aggregation of ribosomal subunits and protect them against dissociation.

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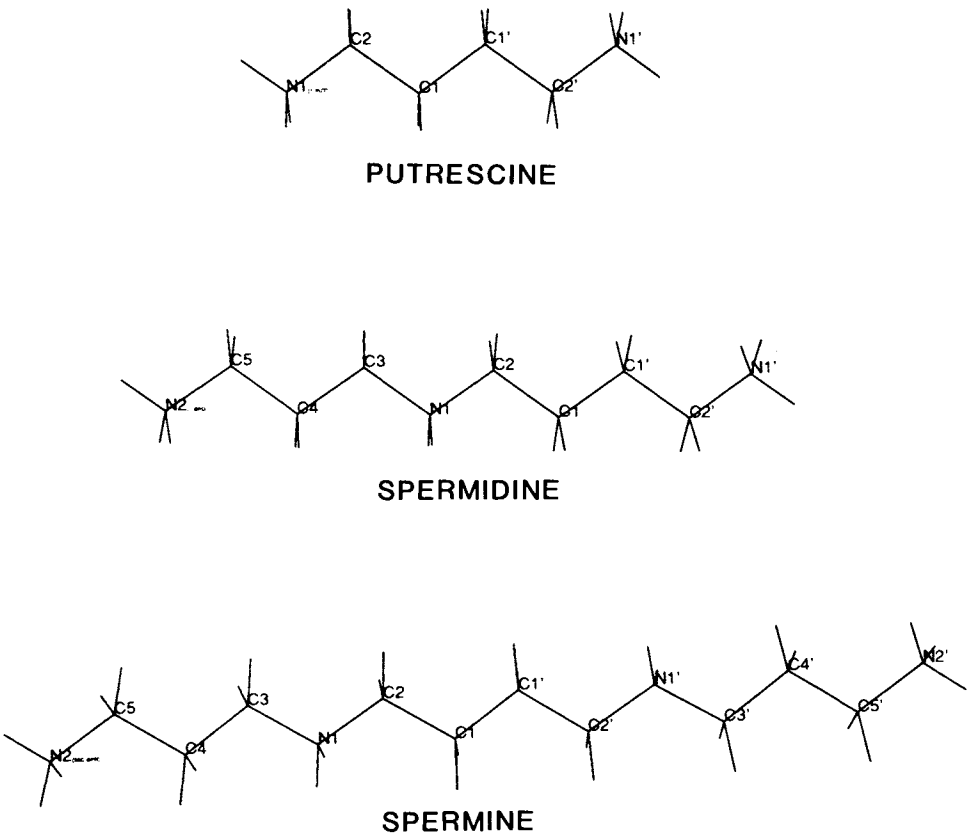

Fig. 1. Chemical structures of the polyamines.

More recent studies have focused on the nature of the association between polyamines and nucleic acids. Initial biochemical studies by Cohen and his co-workers (6–8) indicated that polyamine binding to *t*RNA was specific. They postulated that this binding stabilized *t*RNA secondary and tertiary structure. More recently, crystallographic studies of $tRNA^{phe}$ indicate that two spermine molecules are found at specific sites and do appear to stabilize both the secondary and tertiary structure (9). One spermine is found in the major groove of the double helix and extends into the anticodon stem from one end of the D stem. It appears to bond to four phosphate oxygens in such a way that the opposite sides of the major groove are drawn together; the *t*RNA strands in this region are approximately 3 Å closer than they would be in the absence of spermine. Spermine binding helps produce a 25° deviation from colinearity between the axes of the anticodon and D stems, which is very similar to the description of a DNA major groove binding model discussed below. The other spermine in the $tRNA^{phe}$ crystal "is near the variable loop and curls around phosphate 10 in a region where the polynucleotide chain takes a sharp turn" (9).

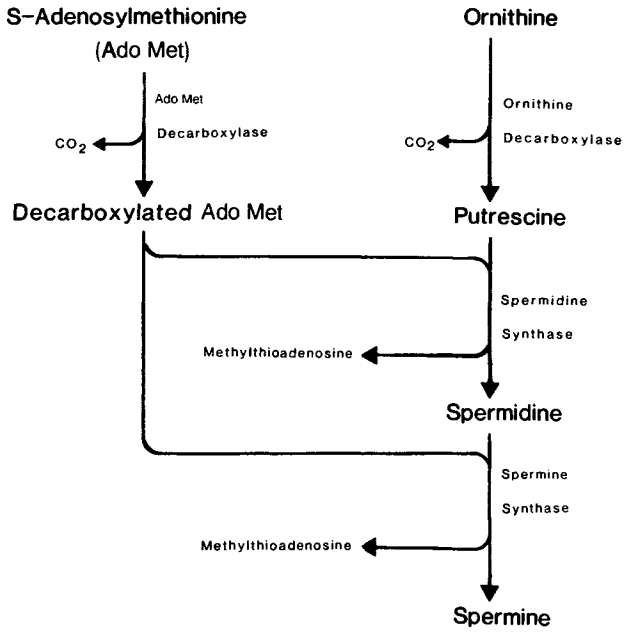

Fig. 2. Biosynthesis of polyamines in eukaryotic cells.

A number of theories and models for polyamine/DNA interactions have been suggested. Bloomfield and Wilson (10) applied counterion condensation theory, which models DNA as a linear distribution of negative charge and polyamines as point concentrations of positive charge without a specific site of interaction. They found that this theory gave a fairly accurate description of polyamine binding to DNA as a function of salt concentration. However, the values of association constants could not be predicted accurately by the theory, and the authors felt that some degree of "translational localization" or "restriction of internal degrees of freedom" was possible. Site specific models of interaction have been proposed by Tsuboi (11) and by Liquori *et al.*

(12). In the Liquori model, the tetramethylene chain portion of any of the polyamines spans the minor groove of B-DNA and the positively charged amine residues are in proximity to the negatively charged phosphate residues on opposite DNA strands. This model for spermine, in which the primary terminal amines interact with a phosphate oxygen on the same side of the minor groove as the adjacent secondary amine, is shown in Fig. 3. A possible flaw in this model becomes apparent when computerized graphic modeling techniques are applied (13) and the complex is viewed from the side (Fig. 4). A space is formed in the minor groove under the tetramethylene bridge of spermine; because the central tetramethylene portion of spermine is hydrophobic, it would be energically unfavorable to fill the space beneath it in the minor groove with water molecules.

Drew and Dickerson (14) published X-ray diffraction data for a double-stranded B-DNA dodecamer d(CGCGAATTCGCG) that, when crystallized in the presence of spermine, bound one spermine molecule per dodecamer in the major groove. Based on these data, we evaluated a major groove binding model for spermine (13). Using computerized graphic modeling and energy minimization techniques, we showed that placing spermine in the major groove of B-DNA heteropolymers (Fig. 5) generated changes in DNA conformation. These changes are the result of electrostatic and hydrogen bonds between the polydeoxynucleotide and spermine. Negative electrostatic potential energy on the polynucleotide tends to concentrate near the positively charged protonated spermine in such a way that electrostatic complimentarity is produced. The complex exhibits this association structurally by maximizing interactions between proton acceptors on the polynucleotide and proton donors on spermine, each amine on spermine interacting with either Z phosphate oxygens or a phosphate oxygen and an N_7 purine site (Fig. 6). These interactions result in the formation of a bend in the polynucleotide in which the major groove of DNA folds over, forming a pocket to enclose the spermine molecule. To accommodate spermine, the remaining portion of the DNA molecule also changes conformation. The minor groove opens widely, the sugar conformations change, and both intra- and interchain phosphate distances are altered. This model may have implications for polyamine-induced changes in DNA conformation such as toroid formation and both the B- to Z-DNA and the B- to A-DNA transformations. In addition, the bend is similar to that described above in the anticodon loop of a tRNA^{phe} crystal.

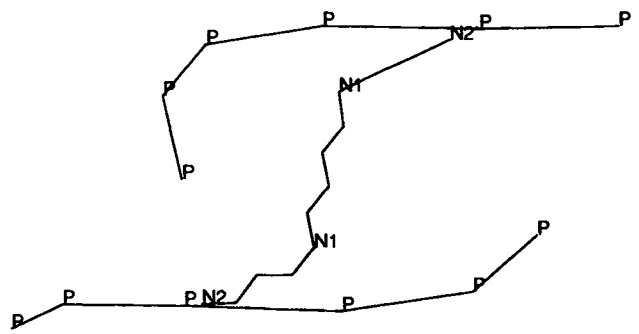

Fig. 3. Tsuboi/Liquori model. Schematic drawing of spermine spanning the minor groove of DNA. Coordinates were taken from Ref. 12.

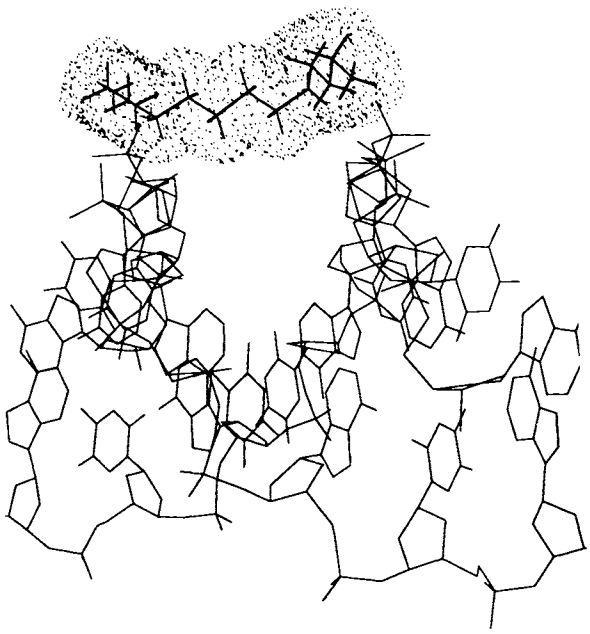

Fig. 4. Tsuboi/Liquori model. A space is produced in the minor groove under spermine. (Spermine is shown with its van der Waal surface.)

It is likely that both nonspecific and specific interactions between polyamines and DNA are of importance, with a series of specific interactions superimposed on a background of nonspecific but highly regulated cationic function. We have recently reviewed available evidence for such specific interactions (4,5) and note here briefly that the ability of the polyamines to condense DNA into toroidal structures and to facilitate DNA conformational changes, especially the change from B- to Z-DNA, appears to involve specific interactions. For instance, a spermine-to-nucleotide ratio of 1:40–50 is adequate to effect the transition of B- to Z-DNA in poly(dG-m^{5}dC) (15,16). This fact is not easily explained by nonspecific interactions. The involvement of the polyamines in both toroidal condensation and B–Z transition has implications both for the organization of nucleic acids and for the regulation of DNA function. The intimate involvement of the polyamines in cellular growth clearly may relate to these nucleic acid interactions, although many other mechanisms can and have been implicated (4).

POLYAMINES IN CANCER THERAPY

The initiation of both *in vivo* and *in vitro* mammalian cell growth is accompanied by an increase both in the activity of the polyamine biosynthetic enzymes and in intracellular polyamine concentrations. Although it is not known whether polyamine accumulation regulates the initiation of cell growth or whether, once initiated, cells require polyamines to maintain growth, it is clear that if polyamine biosynthesis is interrupted, growth is either slowed or stopped; in some circumstances, cell death occurs. Because neoplastic cells grow more rapidly than their normal counterparts, polyamine biosynthetic pathways are obvious targets for cancer therapy.

The concept of using cytotoxic drugs to kill tumor cells

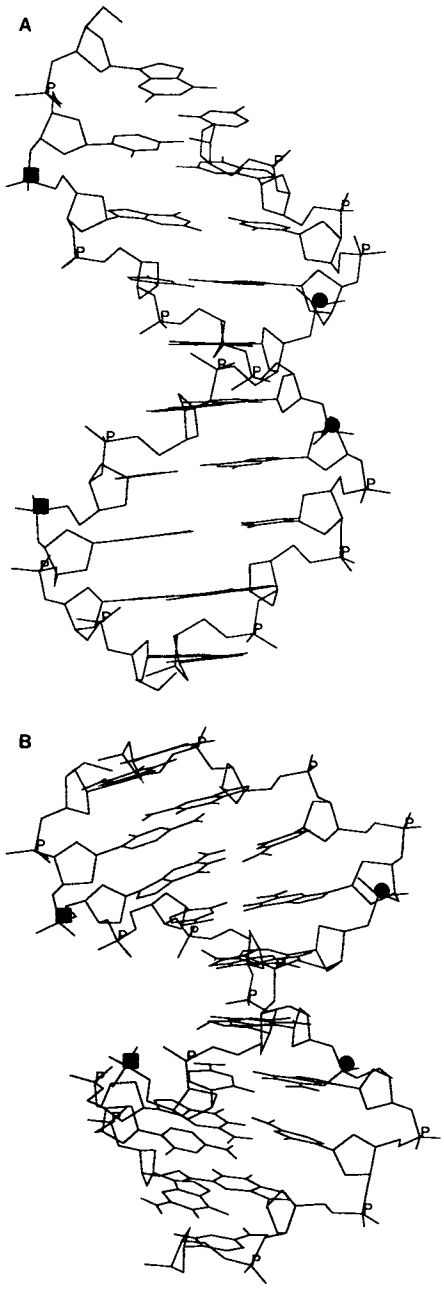

Fig. 5. Major groove model of DNA. (A) B-DNA without spermine. The major groove is on the left (■). Note the smaller size of the minor groove on the right (●). (B) Change in DNA conformation after energy minimization in the presence of spermine (not shown for clarity). Note that the major groove (■) on the left is much narrower than in A, while the minor groove (●) is much wider. The DNA is poly(dG-dC).

has been used clinically for many years. Other, more subtle, approaches are being considered as possible alternatives or adjuncts to traditional therapy. The use of relatively nontoxic agents to diminish or prevent tumor repopulation between courses of cytotoxic therapy and the use of one agent to potentiate the cytotoxic effects of another agent are two approaches that we have discussed elsewhere relative to polyamine biosynthesis inhibitors (17) and which we are presently investigating clinically.

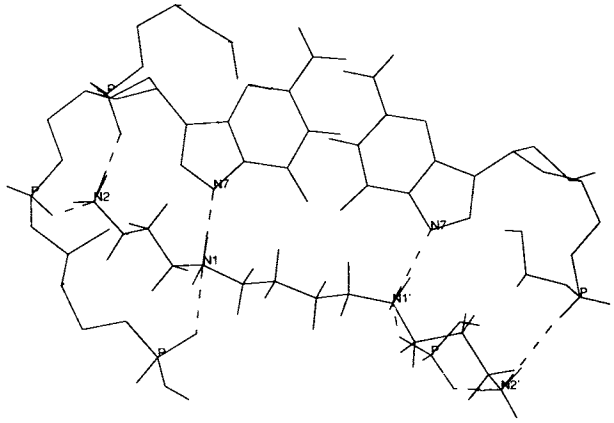

Fig. 6. Spermine interactions with DNA in the major groove. The nitrogens on spermine (N_2, N_1, N_1', N_2') interact with phosphate oxygens and N_7's on guanine. Interactions are shown by dashed lines. The view is along the Z axis of DNA.

A variety of compounds that inhibit polyamine biosynthesis, primarily by interfering with ODC or AdoMetDC, have been synthesized. Inhibitors of the polyamine synthases (18,19) and a variety of polyamine analogues that might interfere with either biosynthesis or function are being developed (20). α-Difluoromethylornithine (DFMO), an irreversible ODC inhibitor (21), has been studied extensively not only as a therapeutic agent but also as a tool with which to probe polyamine biochemistry and function. DFMO usually depletes cells of putrescine and spermidine, with little or no effect on spermine. In most *in vivo* and *in vitro* models in which it has been studied, DFMO causes little or no cytotoxicity; growth is inhibited or halted while cells remain viable. However, DFMO is cytotoxic to human small cell carcinoma (22), rat 9L tumor cells in spheroids (23), and murine B16 melanoma (24).

Methylglyoxal-*bis* (guanylhydrazone) (MGBG), a spermidine analogue that is a competitive inhibitor of AdoMet DC, effectively depletes cells of spermidine and spermine in most experimental systems studied. The cytotoxic effects of MGBG, however, may not relate entirely to inhibition of polyamine biosynthesis (25); the relation between cytotoxicity and polyamine depletion not being direct. Nevertheless, because MGBG is a structural analogue of spermidine, its ability to replace spermidine at certain sites in a cell must be considered in the evaluation of its mechanism of action. MGBG is currently the subject of renewed clinical trials.

None of the inhibitors presently available totally deplete cells of polyamines. In fact, inhibition of one step of the pathway may cause an increase in the concentration of another polyamine either before or after the site of the block. The fact that many polyamine inhibitors are frequently not cytotoxic very possibly is related to this incomplete inhibition. It is likely that an inhibitor or a combination of inhibitors capable of depleting all polyamines would be cytotoxic.

Inhibition of polyamine biosynthesis and depletion of polyamines represent one approach to interfering with the intracellular function(s) of these compounds. In another approach, naturally occurring polyamines could be replaced with analogues designed to interfere with polyamine func-

tion at their sites of action. Modest *et al.* (26) have synthesized a large number of polyamine analogues and found that they had significant activity against various transplantable tumors. More recently, Porter and Bergeron (20) investigated structural properties of various polyamine analogues and found that specific alterations in chain length allowed some analogues to support growth in polyamine-depleted cells while others did not. Together with the evidence for specificity noted above, these results suggest that it might be possible to design analogues to block normal polyamine function in cells.

EFFECTS OF POLYAMINE DEPLETION ON CYTOTOXIC ACTION OF DRUGS

2-Chloroethylnitrosoureas

In addition to the direct effects of polyamines on cells described above, we have investigated the effects of polyamine depletion on the cytotoxic activity of several DNA-directed chemotherapeutic agents. We found that DFMO-induced polyamine depletion of 9L cells in tissue culture affects the conformation of DNA (26) and reasoned from this result that changing the conformation should affect the reaction of DNA with these chemotherapeutic agents.

The chloroethylnitrosourea 1,3-*bis* (2-chloroethyl)-1-nitrosourea (BCNU) is thought to kill cells by a two-step reaction sequence involving initial alkylation and subsequent cross-linking of DNA (28). We have shown that treatment of 9L cells either in monolayer (29) or as multicellular spheroids (24) with DFMO before BCNU treatment potentiates BCNU cytotoxicity. Polyamine depletion can obviously affect a variety of cellular sites and functions in addition to DNA, however, and the effect of polyamine depletion on BCNU cytotoxicity could be secondary to alterations in cellular functions other than at the level of DNA conformation.

Even though BCNU is classified as a cell cycle-nonspecific agent, it is more cytotoxic to 9L cells in the G_1 and G_2/M phases than in the S phase of the cell cycle (30). Therefore, if DFMO caused cells to accumulate in one of the sensitive phases, BCNU cytotoxicity would be expected to increase. DFMO treatment does not have such a cell cycle effect in 9L cells (31), but other cells lines are known to accumulate in different phases of the cell cycle after treatment with polyamine biosynthesis inhibitors (32–35). The possible presence of cell cycle effects should always be considered in studies of the effects of polyamine biosynthesis inhibitors.

We studied the effects of DFMO pretreatment followed by a 1-hr treatment with BCNU on the induction of sister chromatid exchanges (36), a measure of damage to chromosomes, and the formation of DNA cross-links in 9L cells (37). Both measures of DNA damage increased with DFMO pretreatment compared to BCNU alone. Moreover, these effects could be prevented if exogenous putrescine were added to the culture medium to restore intracellular polyamine concentrations before treatment with BCNU.

Because BCNU monoalkylates and carbamoylates DNA in addition to forming cross-links, it is important to know whether alkylation or carbamoylation reactions are modified by DFMO pretreatment. Using a series of nitrosoureas that primarily carbamoylate, alkylate, or cross-link DNA, we found that polyamine depletion had no effect on cytotoxicity caused by monoalkylating agents, inhibited the cytotoxicity caused by a carbamoylating agent, and potentiated the cytotoxicity caused by the cross-linking agent in 9L cells (38). Using a ^{14}C-labeled chloroethylnitrosourea, we also showed that monoalkyl products were not increased by polyamine depletion in 9L cells (39). These and other data show that cross-linking is probably the cytotoxically important step in mechanisms of BCNU cell kill that are affected by polyamine depletion. These findings correlate well with the known effects of polyamine depletion on DNA conformation. Results of preliminary computer modeling studies of these effects are discussed below.

Polyamine depletion might also affect DNA repair mechanisms because a decrease in repair activity could increase the cytotoxicity of a specific drug. A preliminary study in 9L cells indicates that repair in this cell line is not modified by DFMO treatment (39). In a recent study of the effects of DFMO on the cytotoxicity of L-phenylalanine mustard in a human Burkitt's lymphoma cell line, however, a DFMO-induced delay in the appearance of a cross-link repair system was postulated to account for at least part of the increased cytotoxicity observed (40). In a study of the effects of DFMO on the topoisomerase II-mediated DNA scission produced by m-AMSA, it was shown that protein-concealed and protein-associated DNA scission was increased by DFMO pretreatment and that the effect was partially prevented when exogenous putrescine was added after DFMO depletion but before treatment with m-AMSA (41). Further study of the effects of polyamine depletion on DNA scission and repair mechanisms is obviously necessary in a variety of cell lines.

We have been able to potentiate the cytotoxicity of BCNU in 9L cells with MGBG (42) in a manner virtually identical to that shown for DFMO, despite the fact that these two polyamine biosynthesis inhibitors have very different effects on polyamine concentration; putrescine and spermidine are depleted and spermine is virtually unaffected by DFMO, while MGBG depletes spermidine and spermine and mildly elevates putrescine. If dicyclohexylamine (DCHA)[4] is used to inhibit spermidine synthase in 9L cells, putrescine concentrations are markedly increased, spermidine concentrations are significantly decreased, and spermine concentrations are essentially unaffected (43). Pretreatment of 9L cells with DCHA before BCNU treatment does not potentiate BCNU cytotoxicity (43). Experiments using DFMO plus putrescine indicate that the large accumulation of putrescine caused by DCHA counteracts the spermidine depletion caused by the same agent. Thus, the use of inhibitors of polyamine biosynthesis is complicated not only by the specific polyamine(s) depleted, but also by the polyamine(s) that may be elevated secondary to an enzymatic block. In addition, because we currently have little information regarding the intracellular compartmentalization of the polyamines, we cannot relate the change in total intracellular polyamines to specific sites of action.

[4] Recent evidence indicates that cyclohexylamine rather than dicyclohexylamine is the active compound (49).

cis-Platinum

The cytotoxicity of *cis*-diamminedichloroplatinum (II) (*cis*-platinum) is decreased by DFMO pretreatment of 9L cells (44). Damage to chromosomes (36) and the amount of DNA cross-linking (39) were also decreased, and the addition of exogenous putrescine prevented these effects. Thus, polyamine inhibition by the same agent in the same cell line alters chemosensitivity to two cross-linking agents quite differently.

Possible Mechanisms for the Effects of Polyamine Depletion on the Actions of Drugs.

Consideration of the molecular mechanisms of cross-linking reactions for both BCNU and *cis*-platinum on DNA shows that the different results obtained with the two agents may be internally consistent. After the initial metalation reaction, *cis*-platinum is confined to forming a cross-link across the 3.3-Å distance that separates its reactive groups from DNA base nucleophiles on the opposite strand. Thus, any change in the spatial separation or orientation of DNA nucleophiles will affect the formation of *cis*-platinum cross-links. In our computerized graphic modeling studies of the spermine–dodecamer crystal structure discussed above, we noted that the distances by which bases rise in the helix at the end of this molecule that binds spermine—even though it is symmetric, the dodecamer binds spermine at only one end—is in the range of the expected 3.4 Å, while the distance at the other end is greater (unpublished data). Thus, when polyamines are depleted the *cis*-platinum molecule may not be able to form the cross-link. In contrast, the 2-chloroethyl alkylation product of BCNU is more flexible than the DNA–platinum complex, and a change in DNA conformation may favor the displacement reaction necessary for formation of the ethyl cross-link. It is also possible that BCNU may not be able to cross-link DNA easily across areas that are normally occupied by polyamines. Preliminary modeling studies of the spermine/DNA major groove model indicate that formation of the 1-(O^6-guanyl)-2-(O^6-guanine)ethyl cross-link would be very unfavorable at the site of polyamine binding (unpublished results). (These results suggest a possible conceptual difference: it is not that polyamine depletion *increases* cross-linking, but that polyamine binding to DNA *prevents* cross-linking.) Although only spermine has been used in our modeling studies on DNA, the other polyamines may also interact with DNA in the major or minor groove.

EFFECTS ON TREATMENT PROTOCOLS

These results show that polyamine depletion affects the cytotoxicity of different agents in different ways and, therefore, may confer a therapeutic advantage in one case and a therapeutic disadvantage in another. This inference applies only when the drug sequencing is as described (the cytotoxic agent following polyamine depletion). It is quite possible that a polyamine biosynthesis inhibitor given with another therapeutic agent either at the same time or in the reverse sequence (the polyamine biosynthesis inhibitor afterward) may produce significantly different outcomes. Certainly the ability of a polyamine biosynthesis inhibitor to delay growth may confer a therapeutic advantage in preventing repopulation of the tumor following the effects of a cytotoxic agent (18). Thus, giving DFMO with *cis*-platinum in a different sequence may be advantageous.

Using the murine glioma 26 and the 9L rat intracerebral tumors *in vivo*, we investigated the ability of DFMO pretreatment either to potentiate the cytotoxicity of BCNU or to delay tumor repopulation by treating with DFMO after BCNU (45). Survival was increased significantly using either approach. A clinical trial is presently under way using DFMO both before and after BCNU to capitalize on both effects in patients with recurrent gliomas. In designing clinical trials using polyamine biosynthesis inhibitors in combination protocols, it is essential that correct sequencing and dosing be considered to maximize tumor cell kill and to minimize normal cell toxicity. Many of the effects of polyamine biosynthesis inhibitors may also be applicable to rapidly proliferating normal tissues.

In addition to the interactions reviewed in this paper, polyamine biosynthesis inhibitors can be combined with other agents to capitalize on mechanisms that do not directly involve DNA. These include DFMO together with cell cycle-specific agents such as cytosine arabinoside (33), DFMO with MGBG (45,46), a combination that takes advantage of competing transport between spermidine and MGBG, and DFMO with interferon (24). In addition to studies related to cancer, the recent use of polyamine biosynthesis inhibitors in parasitic disease (48) is an exciting approach that should yield new insight into intracellular molecular mechanisms related to the polyamines.

ACKNOWLEDGMENTS

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