Recent work has shown that mitoxantrone can be activated by formaldehyde in vitro to form DNA adducts that are specific for CpG and Cpa sites in DNA. The CpG specificity of adduct formation prompted investigations into the effect of cytosine methylation (CpG) on adduct formation, since the majority of CpG dinucleotides in the mammalian genome are methylated and hypermethylated in subsets of genes is associated with various neoplasms. Upon methylation of a 512-base pair DNA fragment (containing the lac UV5 promoter) using HpaII methylase, three CCGG sites downstream of the promoter were methylated at C5 of the internal cytosine residue. In vitro transcription studies of mitoxantrone-reacted DNA revealed a 3-fold enhancement in transcriptional blockage (and hence adduct formation) exclusively at these methylated sites. In vitro cross-linking assays also revealed that methylation enhanced mitoxantrone adduct formation by 2–3-fold, and methylation of cytosine at a single potential drug binding site on a duplex oligonucleotide also enhanced adduct levels by 3-fold. Collectively, these results indicate preferential adduct formation at methylated CpG sites. However, adducts at these methylated sites exhibited the same stability as nonmethylated sites, suggesting that cytosine methylation increases drug accessibility to DNA that is rather than being involved in kinetic stabilization of the adduct.

Mitoxantrone is a synthetic anticancer agent that is a member of the anthracenedione class of compounds and was originally designed as a simplified analogue of the anthraquinone-containing anthracyclines (1, 2). Because of the similar cytotoxicity to the anthracyclines yet lack of associated cardiotoxicity (3, 4), mitoxantrone is an important anticancer agent and has good activity against solid tumors and myeloid cancers (5).

Recently, we have found that formaldehyde facilitates the formation of mitoxantrone-DNA adducts and that these adducts stabilize duplex DNA sufficiently to prevent strand separation in in vitro cross-linking assays (6). This structure was unstable and heat-labile, therefore not representing a classical covalent cross-link, and like Adriamycin adducts these structures have been termed “virtual cross-links” (6). The possible potentiation of mitoxantrone cytotoxicity in cultures of formaldehyde may provide an explanation as to why mitoxantrone is most effective against myeloid cancers, since they are known to have higher levels of formaldehyde (7). We have also shown that these formaldehyde-activated mitoxantrone-DNA adducts form predominantly at CpG and Cpa sequences (8). The CpG specificity of adduct formation in vitro suggested that methylation may modulate adduct formation in cells. It is known that CpG dinucleotides are underrepresented in the genome with significant depletion of the doublet in ~99% of the genome in higher eukaryotes, whereas the remaining 1% contains CpG at the expected frequency (9, 10). In mammalian species, these CpG dinucleotides are known to be highly methylated with 60–90% methylation at the 5-position of cytosine, whereas only 3–5% of isolated cytosine residues occur as 5-methylcytosine (9, 10). Control of the methylation status of cytosines plays a central role in regulating expression of genes during mammalian development (11). In contrast, tumor cells often have aberrant DNA methylation, and it has been hypothesized that altered methylation is an important component of neoplastic transformation (10). Past studies have reported hypomethylation of the genome and of specific genes in human tumors when compared with noncancerous cells (12–14). The demethylation appears to be random, because specific genes that presumably would not contribute to tumor progression (not involved in cell proliferation and suppression) have been found to be hypomethylated (10).

However, it has also been reported that there is an increase in methyltransferase activity and regions of hypermethylation in some cancer cell lines. These findings are surprising considering the widespread hypomethylation of DNA in tumors. In some cell lines, DNA methylase activity has been found to be abnormally high, with a ~30–50-fold increase in virally transformed cells and a several hundred-fold increase in human cancer cells (15). However, this is not accompanied by increased global methylation (16). Baylin et al. (12) have examined methylation of the calcitonin gene in human lymphoid and acute myeloid malignancies in both cultured and uncultured tumors and detected increased methylation at CCGG sites in the 5’ region of the gene. An increase in methylation was found in 90% of patients with non-Hodgkin’s lymphoid neoplasms and in 95% of tumor cell DNA extracted from patients with acute nonlymphocytic leukemia. These unusual methylation patterns in the calcitonin gene were found much less frequently in other tumor types (12, 13). More recently, Clark and colleagues (17) analyzed the methylation pattern of a number of tumor-related genes in leukemia and found that 90% of acute myeloid leukemia patients exhibited an abnormal methylation pattern with hypermethylation in at least one gene, and 75% exhibited an increase in methylation in two or more of the target genes. This was in contrast to the normal control samples, which were essentially unmethylated. Hypermethylation in leukemia is therefore not limited to single genes (17). In contrast, a recent global analysis of the methylation status of 1200 CpG islands in 98 primary human tumors found that some tumors (including breast and testicular tumors) dis-
played relatively decreased levels of methylation; however, others (including myeloid leukemias) displayed a higher frequency of methylation (18).

Since mitoxantrone-induced DNA adducts have been found to occur preferentially at CG sites, which are the dinucleotide substrates of methylation, and because significant change in methylation status accompanies neoplasia, we aimed to establish whether methylation modulates the ability of mitoxantrone to form adducts with CpG sequences. The ultimate objective is to provide insight into the mechanism of adduct formation at methylated DNA sites in tumor cells and to establish how the extent of methylation of DNA contributes to the sensitivity of cells to mitoxantrone.

**EXPERIMENTAL PROCEDURES**

**Materials—**Mitoxantrone was kindly provided by Lederle Laboratories (Pearl River, NY). The methylases (*Hpa*II, *Msp*I, and *Sss*I) and T4 polynucleotide kinase were obtained from New England Biolabs. NTPs, 3′-O-MeG, 3′-O-MeC, 3′-O-PPPATP, 3′-O-DDPdCTP, 3′-O-UTP (3000 Ci/mmol), ribonuclease inhibitor (RNA guard, human placenta), *Escherichia coli* RNA polymerase, and ProbeQuant G-50 microcolumns were purchased from Amersham Pharmacia Biotech. The nonmethylated and methylated 20-mer oligonucleotides were obtained from GeneWorks and Sigma Genosys, respectively. Acrylamide and molecular biology grade urea were obtained from ICN Biomedicals Inc. Bisacrylamide, ammonium persulfate, and micro Bio-spin 1 chromatography column. The self-complementary oligonucleotides were resuspended in transcription reaction mix. Mitoxantrone was kindly provided by Lederle Laboratories (Pearl River, NY). The methylases (*Hpa*II, *Msp*I, and *Sss*I) and T4 polynucleotide kinase were obtained from New England Biolabs. Glyoxen was from Applied Molecular Biochemicals and Centricron TM-3 columns were from Millipore Corp. (Amicon Bioseparations). Plasmid purification kits were purchased from Qiagen, and formamide was from Sigma. Kall thymus DNA was from Worthington, and Ready Safe cell lysis buffer was purchased from Beckman. All other chemicals and reagents were of analytical grade, and all solutions were prepared using water from a Milli-Q (Millipore Corp.) four-stage purification system.

**DNA Isolation and Methylation—**The plasmid pC1 (19) was isolated using a Qiagen Plasmid Maxi Kit. For transcription assays, the plasmid was restriction-digested with *Pvu*II and *Hind*III to isolate a 252-bp fragment (containing the *lac UV5* promoter), which was purified by electroelution. A portion of this DNA was reanimated with *Hpa*II methylase in the presence of 80 μM S-adenosylhomocysteine at 37 °C for 1 h. This specifically methylated the CG cytosine (5′-C) of 5′-CCGG sequences. To specifically methylate the first cytosine of the CCGG site, the 5′-12-bp fragment was reacted in the same way with *Msp*I methylase.

It should be noted that the use of *Sss*I methylase (which methylates all CG sites) resulted in the total loss of initiation of transcription and hence could not be used for the transcription assay.

For *in vitro* cross-linking assays, the pC1 plasmid was linearized by restriction digestion with *Sal*I to yield a 3496-bp DNA fragment. The DNA was 3′-end-labeled as described previously (6) and treated with *Sal*I methylase and 80 μM S-adenosylmethionine at 37 °C for 1 h to specifically methylate the 5′-C of cytosines at CG sites.

To confirm methylation status, DNA samples were restriction-digested with *Hpa*II, which recognizes the CCGG site in DNA. Upon methylation of either cytosine, this sequence is not cleaved, and hence DNA remains uncut. In all experiments, methylation levels were >90%.

**Transcription Assay—**The 512-bp DNA fragment was reacted with mitoxantrone and formaldehyde prior to removal of unreacted formaldehyde by ethanol precipitation. The transcription assay was then performed as described previously (8, 20). The RNA transcripts were resolved on a 12% denaturing acrylamide gel preheated to 50°C, and subjected to electrophoresis at 2000 V in TBE buffer.

**Cross-linking Assay—**Renatured DNA (due to formation of mitoxantrone-DNA adducts) was separated from unreacted, denatured DNA as previously described (6), using a 0.8% agarose gel in TAE buffer overnight at 40 V. The gel was vacuum-dried on a Bio-Rad model 583 gel drier prior to exposure to a phosphor screen overnight and then analyzed using a model 400B PhosphorImager and ImageQuant software (Molecular Dynamics, CA).

*1 The abbreviations used are: TEMED, N,N,N′,N′-tetramethylethylene diamine; bp, base pair(s)"
μm bp) samples were treated with 20 μM 14C-labeled mitoxantrone and 3 mM formaldehyde for times up to 180 min at 37 °C. Samples were extracted twice with phenol and once with chloroform prior to ethanol precipitation and resuspension in 50 ml of TE buffer. Scintillation fluid (1 ml) was added to 50-ml samples, and 14C counts were determined using a Wallac 1410 liquid scintillation counter.

RESULTS

Effect of CG Methylation on Transcriptional Blockage—When E. coli RNA polymerase encounters a mitoxantrone-DNA adduct, elongation of the RNA chain is blocked, producing truncated transcripts rather than the 379-base full-length transcript that would be produced if no blocks were encountered. The dependence of this blockage on formaldehyde concentration is illustrated in Fig. 1A. Blockages were dependent on the presence of both formaldehyde and mitoxantrone (control lanes, which lack either of these compounds, yield only full-length transcripts). When DNA was methylated with HpaII methylase, the CG cytosines of CCGG sequences were specifically methylated (CC*GG), and these sites (indicated by the arrows in Fig. 1B) revealed a greater intensity of transcriptional blockages compared with the corresponding unmethylated sites. When methylated DNA was incubated with mitoxantrone alone, there were no transcriptional blockages, indicating that formaldehyde is required for adduct formation and also that methylation itself does not cause stalling of RNA polymerase.

The extent of blockages at all methylated sites was quantitated as a percentage of the total amount of transcripts in each lane (Fig. 2). Fig. 2, A–C, illustrates the increase of adduct formation when the cytosine residue of CG sites was methylated. There is at least a 2-fold increase in block intensity at methylated sites, indicating that methylation enhances mitoxantrone-DNA adduct formation. Fig. 2, D–F, shows controls that represent increasing block intensity with formaldehyde concentrations at sites that have not been methylated. These images show that there was no significant difference of adduct formation at unmethylated sites on the two DNA templates, thus confirming that the enhanced adduct levels at methylated CG sites is a specific consequence of methylation of cytosine residues.

Effect of C*CGG Methylation on Adduct Formation—Since it was known that mitoxantrone was CG-specific and methylation of these sites led to enhanced adduct formation, it was of interest to determine the effect of methylation of adjacent cytosine residues. DNA was treated with MspI methylase to specifically methylate the first cytosine of the CCGG DNA sequence (denoted as C*CGG). The dependence of formaldehyde on transcriptional blockage with unmethylated (Fig. 3A) and methylated DNA (Fig. 3B) was compared. All CCGG sites are indicated by arrows, and in contrast to HpaII methylation, there was no enhancement of block frequency at the methylated sites. The extent of blockages at these sites and other non-CCGG sites (that do not become methylated) was quanti-
tated as a percentage of the total amount of transcripts in each lane. Fig. 3C shows a comparison of the block frequency at methylated (●) and unmethylated (■) CCGG sites. The mole fraction of block 16 was calculated and plotted as a percentage of transcripts in the entire lane at the varying formaldehyde concentrations. D, comparison of block frequency at a non-CCGG site (block 7) that has not been methylated.

**Table I**

| Block | Site   | $t_{1/2}$ -Me | $t_{1/2}$ +Me |
|-------|--------|---------------|---------------|
| 10    | ACGC   | >180          | >180          |
| 11    | CCGG   | 143 ± 37      | 165 ± 60      |
| 13    | TCCT   | 118 ± 21      | 133 ± 18      |
| 17    | CCGG   | 156 ± 65      | 193 ± 40      |

formed, as expected at this nonmethylated site.

**Stability of Adducts at Methylated Sites**—The pronounced enhancement of adduct formation at DNA sequences treated with HpaII methylase prompted an investigation of the stability of these adducts. DNA was reacted with mitoxantrone and formaldehyde to yield a high level of adducts at each CG site. Adduct stability was assessed at 37 °C for elongation times up to 180 min for both unmethylated and methylated DNA (Fig. 4, A and B, respectively). First-order kinetic analysis of the loss of blockages at individual sites revealed that there was no significant difference between the loss of transcriptional blockages at CG and C*G sequences. The half-lives for the loss of adducts at a number of blockage sites are summarized in Table I.

Although it was found that methylation using MspI methylase did not enhance adduct formation, studies were undertaken to determine whether methylation of the first cytosine in the CCGG sequence altered the stability of transcriptional blockages. The stability at these sites was assessed as described above, and there was no significant difference in stability between CCG and C*CG sites.

**Effect of Methylation on Mitoxantrone Adduct Formation Using a Cross-linking Assay**—When DNA is treated with mitoxantrone and formaldehyde, it has been shown using in vitro cross-linking assays that the resulting adducts stabilize DNA sufficiently to resist strand separation. Cross-link formation was shown to be dependent on formaldehyde concentration for both unmethylated DNA (Fig. 5A) and DNA that was methyl-
Methylation Enhances Mitoxantrone DNA Adducts

**DISCUSSION**

**Enhanced Adducts at Methylated CG Sites**—Our recent studies demonstrated that mitoxantrone-DNA adducts blocked transcription at specific sites in DNA (8). These sites included CpA and CpG sequences, with the latter showing a slightly enhanced stability at 37 °C. The specificity of binding to CpG sites prompted an investigation into the effect of cytosine methylation on adduct formation at these sites. The underrepresentation of this doublet in the genome, but known high frequency sites prompted an investigation into the effect of cytosine methylation on adduct formation at these sites. The underrepresentation of this doublet in the genome, but known high frequency...

![Diagram](http://www.jbc.org/)

**FIG. 5.** Dependence of cross-link formation on methylation of CpG sites. A, end-labeled DNA (25 μm bp) was incubated with 20 μM mitoxantrone and 0–3 mM formaldehyde (as shown) in PBS at 37 °C for 2 h. The samples were extracted once with phenol and chloroform and then ethanol-precipitated. The DNA was resuspended in TE buffer and denatured at 60 °C for 5 min in 45% formamide loading buffer. The samples were then subjected to electrophoresis on a 0.8% agarose gel at 45 V overnight. Lane C, a control lacking mitoxantrone but containing 3 mM formaldehyde. B, end-labeled DNA was treated with SssI methylase to specifically methylate CG sites in DNA. The DNA was extracted once with phenol and chloroform and precipitated with ethanol prior to reaction as in A. C, quantitation of the gels shown in A and B. The percentage of total DNA containing interstrand cross-links was calculated and expressed as a function of formaldehyde concentration for methylated (●) and unmethylated (■) DNA.

...ated at the C-5 cytosine of CG sequences using SssI methylase (Fig. 5B). Quantitation of renatured DNA (Fig. 5C) revealed a significant increase in adduct formation upon methylation of CG sites, with at least a 3-fold increase in adduct levels at concentrations below 2 mM formaldehyde.

Although there was no change in the stability of adducts detected as transcriptional blockages, it was of interest to determine whether methylation enhanced the stabilization of DNA with respect to strand separation. Fig. 6, A and B, reveals the loss of “virtual cross-links” over time at 37 °C. These results were quantitated (Fig. 6C) and reveal that, as with transcriptional block stability, there was a slight, but not significant, difference in adduct stability following cytosine methylation (20 and 27 min for unmethylated and methylated DNA, respectively).

**CpG Oligonucleotide Methylation Enhances Drug Binding**—To determine the effect of methylation of the cytosine residue of a 20-mer oligonucleotide, two identical oligonucleotides were utilized, one of which contained the C-5 cytosine modification. The formation of oligonucleotide-mitoxantrone adducts stabilizes the DNA duplex such that it migrates essentially as the duplex form following denaturing conditions. The formation of these duplexes was dependent on formaldehyde (Fig. 7), and there was an obvious increase in adduct formation when the 20-mer was methylated (Fig. 7B) compared with the unmethylated control (Fig. 7A). The results were quantitated (Fig. 7C) and reveal that there was a 3-fold increase in the number of stabilized duplex oligonucleotides when the cytosine residue of CpG dinucleotides was methylated.

Although methylation enhanced the formation of mitoxantrone-DNA adducts, it was not expected to alter the stability of the adduct at 37 °C, since this was not observed by either transcription nor in vitro cross-linking assays. Both unmethylated (Fig. 8B) and methylated (Fig. 8A) oligonucleotides were reacted with mitoxantrone and formaldehyde, and the stability of the resulting drug-oligonucleotide adducts was assessed at 37 °C for up to 3 h. The loss of adducts was described by a first-order exponential decay, and the half-lives for both the unmethylated and methylated oligonucleotides were similar (20 and 26 min, respectively).

**Methylation Leads to Faster Reaction Rates**—Since it was found that adduct formation was enhanced ~3-fold at methylated CG sites, it was of interest to determine whether this was due to faster reaction rates compared with nonmethylated DNA. Methylated and nonmethylated plasmid DNA samples were treated with [14C]mitoxantrone and formaldehyde at 37 °C for times up to 180 min. The 14C-drug-DNA adducts were quantitated (Fig. 9) and revealed a faster reaction rate, leading to a large increase in adducts with methylated DNA. Regardless of the methylation status, the maximum amount of DNA-drug adducts was formed within 60 min and resulted in approximately twice as many adducts with the methylated CpG dinucleotides.
Methylation Enhances Mitoxantrone DNA Adducts

Upon methylation of the 512-bp DNA fragment using HpaII methylase, three CCGG sites within the sequence were methylated at C5 of the internal cytosine residue. The transcriptional block frequency at these sites was found to be significantly enhanced (~3-fold), indicating an increase of adduct formation at these methylated sites. This finding was potentially important, since there is a high frequency of methylation at these sites in biological systems, and this is associated with differential localized hypo- and hypermethylation in cancer cell lines (12, 14, 17). Cross-linking assays also revealed that methylation enhanced duplex stabilization due to adduct formation by at least 3-fold at low formaldehyde concentrations and 2-fold at higher concentrations. In order to further analyze adduct formation at a single potential drug binding site and the effect of methylation at this site, an oligonucleotide with a single CpG central site was examined. The 3-fold increase in adduct formation accompanying methylation of the 20-mer confirmed the 2–3-fold increase detected by in vitro transcription and cross-linking assays. 

\[ ^{14}C \text{Mitoxantrone} \] experiments revealed faster reaction rates, leading to a 2-fold increase in adduct formation. A 3-fold increase was not expected, since CpA adducts are detected using this technique and these adducts are not influenced by methylation.

**Stabilization of Adducts at Methylated Cytosines**—The dramatic increase in adduct formation at CC*-GG sites (>200%) prompted studies to determine whether these adducts were preferentially stabilized at methylated sites, compared with nonmethylated sites. Although more adducts were being formed at methylated sites, no significant difference in adduct stability was detected (either at individual transcription block sites or in the single adduct-oligonucleotide complexes); nor was there any detectable increase of half-life of the “virtual cross-link.”

The enhanced adduct formation but lack of increased stability raised the question as to whether the methyl group on cytosine participates in the chemical composition of the drug-DNA adduct or whether it serves to increase the accessibility of mitoxantrone to DNA. Control studies indicated that the methylated C5 of cytosine does not replace the methylene group, which appears to derive from formaldehyde, and is thought to be involved in the covalent linking of the amino group on the side chain of mitoxantrone to the N2 of guanine (6), but other structures cannot be ruled out until chemical analysis of the adduct is possible (currently being investigated). Methylated DNA and mitoxantrone alone did not block the progression of RNA polymerase along the drug-treated DNA template, and this provides evidence that mitoxantrone does not react with this methyl group to form an adduct. The cytosine methylation therefore may facilitate a local structural change to DNA that enhances adduct formation at these methylated sites. It has previously been documented that cytosine methylation alters the local structure of duplex DNA, resulting in a conformational change (9, 21) leading to what has recently been termed E-DNA (22), and it therefore appears that it is this structural change that may enhance the accessibility of mitoxantrone to methylated CpG sites. The structural change may serve to enhance the initial intercalation of mitoxantrone into DNA, causing an increased likelihood of covalent reaction.

A previous study that compared sequence preference of the CG-specific drug mitomycin C for DNA containing either 5-methylcytosine or unmethylated cytosine in random sequence DNA oligomers found that methylation enhanced cross-linking efficiency by up to 2-fold. This difference was attributed to either a local charge effect, rendering the N2 of the reactive guanine more nucleophilic, or to a local conformational change rendering it more accessible (9). A later study by Tomasz and co-workers (23) discovered that CpG methylation of the plasmid pBR322 enhanced mitomycin C cross-linking by 3-fold at low concentrations (5 µM), 2-fold at 10 µM, and less thereafter. This increase was also attributed to a conformational change to the DNA helix or an electronic effect that increases the nucleophilicity of guanine-N2. The enhanced reactivity was also shown to be associated with the CpG methylated cytosine, rather than the C5 methylation of cytosine residues flanking this dinucleotide, and these effects are consistent with our observations of mitoxantrone adduct formation at methylated sites. Additional reactivity at methylated CpG sequences has also been detected with benzo[a]pyrene derivatives (24). It would therefore be of interest to clarify if there is a common reaction mechanism for the binding of these compounds (mitoxantrone, mitomycin C, benzo[a]pyrene) to methylated CpG sequences.

**Biological/Medical Implications**—There is a considerable body of evidence to show that tumor cells display hypomethylation over a majority of the genome. In contrast, there is concurrent DNA hypermethylation of multiple genes in particular neoplasms, including acute myeloid leukemia (17), associated with a general deregulation of the methylation status in...
CpG islands that are usually unmethylated apart from the inactive X chromosome (13). The hypermethylation of genes in leukemia has been shown to be cancer type-specific, with a wide number of other cancer types showing decreased methylation of these same genes (13, 17, 18). The present results provide evidence that CpG methylation may enhance the activity of mitoxantrone. This is of significance, since tumor cells have aberrant CpG methylation patterns that are tumor type-specific. However, since tumor cells generally undergo a global decrease in genomic methylation, this may decrease the cytotoxic effectiveness of mitoxantrone against some tumors. It is important to establish the effect of mitoxantrone on cells with differentially methylated genomic regions, since this knowledge should lead to strategies to increase the effectiveness of mitoxantrone. Alternatively, it is possible that the qualitative shift of methylation toward specific CpG islands in certain tumor cells may serve to enhance the activity of mitoxantrone. This would be possible if there was an increase in local levels of mitoxantrone adducts due to gene-specific hypermethylated regions leading to an increase in adduct stability and/or a qualitative shift of methylation toward specific CpG islands.

Fig. 7. Reaction of mitoxantrone with methylated oligonucleotides. A, the unmethylated 20-mer oligonucleotide (25 μM bp) was incubated for 7 h with 40 μM mitoxantrone and 0–50 mM formaldehyde at room temperature. The samples were loaded onto a 19% denaturing acrylamide gel with 45% formamide loading dye and run overnight at 4 °C and 600 V prior to exposure to a phosphor screen for PhosphorImager analysis. C1 and C2, control reactions containing the oligonucleotide and only mitoxantrone or formaldehyde, respectively. B, the methylated 20-mer oligonucleotide was treated as outlined for A. C, quantitation of gels in A and B. The percentage of duplex DNA was quantitated and expressed as a function of formaldehyde concentration for both methylated (○) and unmethylated (●) oligonucleotides.

Fig. 8. Effect of methylation on oligonucleotide adduct stability. A, unmethylated DNA (25 μM bp) was reacted with 40 μM mitoxantrone and 50 mM formaldehyde for 7 h at room temperature. Samples were subjected to gel exclusion (micro Bio-spin P6) and incubated at 37 °C for times up to 3 h and then run at 4 °C overnight on a 19% acrylamide gel at 600 V. B, methylated DNA was reacted with 40 μM mitoxantrone and 30 mM formaldehyde at room temperature for 7 h. Samples were then treated as outlined for A. C, quantitation of the time-dependent loss of cross-links with unmethylated oligonucleotides at 37 °C. D, quantitation of percentage cross-links for cytosine-methylated oligonucleotides as a function of time at 37 °C.

Fig. 9. Time dependence of adduct formation. Both methylated and unmethylated plasmid DNA were reacted with 20 μM mitoxantrone and 3 mM formaldehyde at 37 °C for up to 180 min. The reaction was terminated by two phenol extractions and one chloroform extraction prior to ethanol precipitation and resuspension in TE buffer. 14C-drug-DNA adducts were quantitated by scintillation analysis. The formation of [14C]mitoxantrone-DNA adducts is shown as a function of reaction time at 37 °C.
greater effect on adduct detection, which could lead to apoptotic destruction of the cell. Given the importance of CpG island methylation on gene transcription, it would be interesting to establish the ultimate effect of adducts at these sites in terms of altered gene expression. Studies are currently being undertaken to determine if there is differential cell sensitivity to mitoxantrone with varying methylation status. It is also of interest to analyze adduct and methylation levels in specific genes to determine if there is a correlation between methylation and drug activity in vivo. A positive correlation between cytotoxicity and methylation patterns could lead to the development of biological markers for potential sensitivity of individual patients to mitoxantrone treatment.

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Cytosine Methylation Enhances Mitoxantrone-DNA Adduct Formation at CpG Dinucleotides

Belinda S. Parker, Suzanne M. Cutts and Don R. Phillips

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