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Retrovirus-Mediated Transfer of the Human O^6-Methylguanine-DNA Methyltransferase Gene into a Murine Hematopoietic Stem Cell Line and Resistance to the Toxic Effects of Certain Alkylating Agents

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ABSTRACT. O^6-Methylguanine-DNA methyltransferase (MGMT) is an important DNA repair protein that plays a key role in cancer chemotherapy by alkylating agents such as carmustine (BCNU) and Dacarbazine (DTIC). Therapy by BCNU and DTIC is reduced by dose-limiting hematological toxicity as a result of low MGMT repair activity in bone marrow cells. In this study, we have constructed a Moloney murine leukemia virus retroviral vector containing the human *mgmt* gene. High-titer retrovirus producer cell lines have been generated. Retroviral-mediated transfer of the human *mgmt* gene into murine multi-potent hematopoietic stem cells, FDCP-1, resulted in the expression of a high level of MGMT activity. In comparison with the control cells that were transduced with the parent vector, the MGMT-expressing clones were considerably more resistant to the cytotoxicity of the methylating agents, such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, *N*-nitroso-*N*-methylurea, and temozolomide, as well as the chloroethylating agents 1-(2-chloroethyl)-1-nitrosourea and BCNU. The protection provided by MGMT could be eliminated by the MGMT inactivator O^6-benzylguanine. Thus, the principal lethal lesions produced by these alkylating agents in the murine hematopoietic stem cells and the MGMT deficiency in these cells can be complemented by retroviral-mediated gene transduction. BIOCHEM PHARMACOL 51;9:1221–1228, 1996.

KEY WORDS. retrovirus; gene transfer; MGMT; drug resistance; alkylating agents; methylation; DNA repair.

Alkylating agents have had good utility as cancer chemotherapeutic substances, although a number of them are also potent mutagens and/or carcinogens. The chemotherapeutic efficacy of this class of agents appears related to their ability to alkylate macromolecules and, in particular, DNA. Treatment of cells or of DNA itself with methylating agents such as MNNG[‖] or MNU results in formation of a series of alkylated nucleotides [1]. Among the alkylated moieties, O^6-alkylguanine plays an important role in cancer chemotherapy since it represents a product of the reaction of DNA with several agents that have clinical utility, e.g. CNU and its derivatives, and DTIC. The O^6-alkylguanine in DNA mispairs with thymine rather than cytosine, causing a GC to AT transition that is responsible for the mutagenic action and that may contribute to cytotoxicity [2].

O^6-Methylguanine and other O^6-alkylguanines in DNA can be repaired by MGMT [3–5]. This repair process which involves the transfer of the methyl group from the O^6-position of guanine in DNA to an internal cysteine residue in MGMT is an auto-inactivating stoichiometric reaction. CNU derivatives, such as BCNU, are cytotoxic because of the formation of DNA interstrand cross-links. Initially, exposure of DNA to CNU leads to the chloroethylation of the O^6-position of guanine, then to an internal cyclization with the N^1 of this base, and finally with the cleavage of the ethyl moiety from the O^6-position to form an alkyl bridge between the guanine and a neighboring cytosine or guanine. Prior to the stage of formation of the interstrand cross-link, the alkylated guanine is repairable by MGMT [6].

The transfection of the *Escherichia coli ada* gene into

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‖ *Abbreviations:* MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; MNU, *N*-nitroso-*N*-methylurea; MGMT, O^6-methylguanine-DNA methyltransferase; O^6-BG, O^6-benzylguanine; CNU, 1-(2-chloroethyl)-1-nitrosourea; DTIC, 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; FBS, fetal bovine serum; DMEM, Dulbecco's modified Eagle's medium; G418, geneticin; PCR, polymerase chain reaction; hGH, human growth hormone; TK, thymidine kinase; and CFU, colony forming unit.

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MGMT-deficient (*mex*$^-$) mammalian cells results in a decreased sensitivity to BCNU [7] and to other alkylating agents [8, 9]. Previous results from this laboratory have demonstrated that the expression of human MGMT in the DNA excision repair-deficient CHO cells could restore cellular resistance to certain *N*-nitroso compounds and prevent cytotoxicity by certain alkylating agents [10, 11]. Other investigators have also shown that MGMT activity in CHO cells does lead to an increased resistance to the cytotoxicity and mutagenicity of monofunctional alkylating agents, and to the induction of sister chromatid exchange [9, 12–16]. Hepatic expression of the *E. coli ada* gene in transgenic mice affords protection against nitrosamine-induced hepatocarcinogenesis [17, 18]. Recently, Dumenco *et al.* [19] have established transgenic mice in which the human MGMT is expressed in the thymus, and have noted their protection against the development of thymic lymphomas after exposure to MNU. Very recently, two groups [20, 21] have reported the successful *in vivo* transduction of the human *mgmt* gene in a retroviral vector into murine hematopoietic cells. After bone marrow reconstitution, the MGMT-transduced mice exhibited an increased resistance to the hematological toxicity caused by BCNU.

Clinically, the failure of continued chemotherapy is associated frequently with either development of resistance to the agent, e.g. enhanced expression of the multidrug resistance gene, *mdrl*, or with unacceptable toxicity to normal hematopoeitic stem cells [22]. Thus, toxicity of chemotherapeutic drugs to bone marrow is often a dose-limiting side-effect in the treatment of cancer patients. Since the dose of the chemotherapeutic agent represents a crucial factor in establishing response in the treatment of advanced melanoma, particularly for the alkylating agents, such as BCNU and DTIC, any effort at decreasing the bone marrow toxicity should prove beneficial.

In previous experiments, transfection of the *E. coli ada* gene into an *ada*-deficient murine multi-potent hematopoietic stem cell line, FDCP-1, resulted in a high level expression of MGMT and a high degree of resistance to the toxic effects of methylating and chloroethylating agents, which strongly suggested that MGMT may provide protection against the hematological toxicity produced by these agents [23]. In the present study, we have developed a retrovirus-mediated system to transfer the human *mgmt* gene into FDCP-1 cells and have studied the protective effect of this transduced gene against the cytotoxic effect of alkylating agents such as MNNG, MNU, temozolomide, CNU, and BCNU.

MATERIALS AND METHODS

Chemicals

MNNG and MNU were purchased from the Sigma Chemical Co. (St. Louis, MO). CNU and BCNU were obtained from Dr. Ven L. Narayanan, Drug Synthesis and Chemistry Branch, National Cancer Institute (Bethesda, MD). O^6-BG was donated by Dr. Robert C. Moschel, Chemistry of Carcinogenesis Laboratory, National Cancer Institute. Temozolomide was obtained from the Schering-Plough Co. (Kenilworth, NJ), through the courtesy of Dr. Joseph Catino. All chemicals were dissolved in water-free DMSO and stored at −80°, except for temozolomide, which was suspended in 2% methylcellulose. Immediately before use, aliquots of the DMSO- or methylcellulose-containing materials were diluted with cell culture medium and added to the cell cultures. In all experiments, the final concentration of DMSO did not exceed 0.2%; at this level, the DMSO did not exert any cytotoxicity. Methylcellulose was also without effect on cell survival.

Cells and Culture

FDCP-1, a murine interleukin-3-dependent multi-potent hematopoietic stem cell line [24], was grown in RPMI-1640 supplemented with 10% FBS (GIBCO BRL, Gaithersburg, MD) and 10% WEHI-3B conditioned medium as a source of interleukin-3. PA317 packaging cells [25] were maintained in DMEM-10% FBS containing 7 mM glutamine. GP+E86 packaging cells [26] were maintained in DMEM-10% NCS (HyClone, Logan, UT) containing 8 mM glutamine.

Retroviral Vector Construction

The full-length human MGMT cDNA was PCR-amplified from a pRSV-MGMT expression vector previously made in this laboratory [10] by a *Hinc* II site-containing forward primer consisting of 22 bases from positions 70 to 91 and a *Bam*H I site-containing reverse primer consisting of 19 bases from positions 710 to 728, according to the published MGMT cDNA sequence [27]. This PCR-amplified MGMT fragment was digested by *Hinc* II and blunt-end ligated into the *Hinc* II site of an hGH expression vector under the transcriptional control of the HSV TK promoter. A 12-mer *Xho* I DNA linker (New England BioLabs, Beverly, MA) was inserted into the *Hind* III site upstream to the TK promoter. A DNA fragment that contained the TK promoter and the MGMT cDNA was then endonucleased from this construct by *Xho* I and *Bam*H I, and cloned into the *Xho*I and *Bam*H I cloning sites of the retroviral vector, pXB/XN, an N2 Moloney murine leukemia virus-derived vector containing a neomycin resistance gene under the control of the viral LTR promoter [28]. pXB/XN was provided by Dr. David Bodine of NIHGR (Bethesda, MD). This newly derived construct containing an internal TK promoter, a neomycin selection marker, and a human MGMT cDNA was named pXB/XN-MGMT (Fig. 1). The sequence of the construct was subsequently verified by DNA sequencing techniques.

Generation of a High-Titer Producer Cell Line

An amphotropic cell line, PA317 (a gift from Dr. David Bodine), was transfected with either pXB/XN-MGMT or the control pXB/XN DNA by a modified calcium phos-

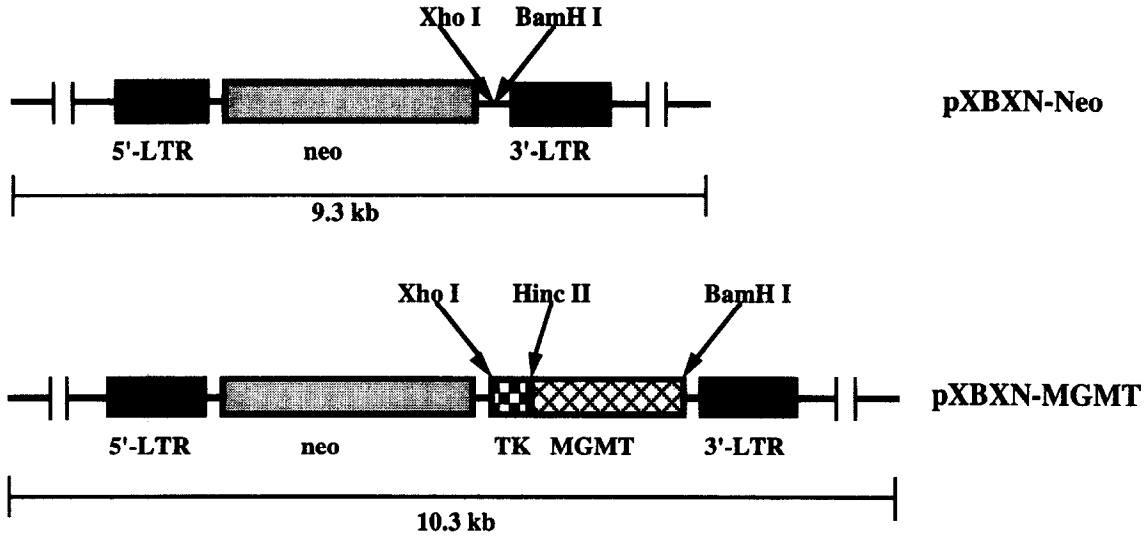

FIG. 1. Retroviral vector containing the human *mgmt* gene, pXB/XN-MGMT. A human MGMT-cDNA was ligated with an HSV-TK promoter, which serves as an internal promoter. This TK-MGMT DNA fragment was then cloned into the *Xho* I and *Bam*H I cloning sites of pXB/XN (pXBXN-Neo in the figure), downstream of the neomycin-resistant gene (*neo*^{r}) driven by the Moloney LTR promoter and enhancer.

phate method [29]. Forty-eight hours later, the medium conditioned by the transfected cells was used to infect the ecotropic cell line, GP+E86, in the presence of 8 μg/mL polybrene (Sigma). The infected cells were selected by G418 (GIBCO BRL) at 1 mg/mL (65–70% of active compound). The G418-resistant clones from pXB/XN-MGMT and pXB/XN transfected cells (named GP+E86-MGMT and GP+E86-Neo, respectively) were isolated and expanded clonally for further characterization. The supernatants produced by these retrovirus producer cell lines after a 24-hr incubation were collected and stored at −80° for later infection.

Retrovirus Titer Assay

The titer of stably transfected retrovirus producer ecotropic cells was assayed on NIH 3T3 cells [30]. NIH 3T3 cells (1×10^5) were plated in 60-mm dishes in DMEM-10% bovine calf serum (HyClone). Twenty-four hours later, the medium was replaced with 5 mL of serial 10-fold dilutions of medium conditioned for 24 hr by confluent cultures of producer cells in the presence of 6 μg/mL polybrene. After an additional 24 hr, the virus-containing medium was replaced with fresh medium. The cells were subjected to G418 selection (0.8 mg/mL) 24 hr later. After 10–12 days, colonies were stained with Giemsa blue (Sigma) and counted.

Retroviral-Mediated Gene Transfer of MGMT into FDCP-1 Cells

FDCP-1 cells (1.0×10^6) in 5 mL of medium were infected with 5 mL of retrovirus-containing supernatant in the presence of 8 μg/mL polybrene. Forty-eight hours later, the cells were selected with 0.8 mg/mL of G418 for an additional 3 days before clonal dilution into 96-well plates for further selection. Individual clones were expanded and tested for MGMT activity.

Cytotoxicity Assay

FDCP cells (1×10^5) were plated into 6-well plates in 3 mL of medium. After overnight incubation, the cells were first treated with or without 10 μM O^6-BG for 1 hr, then exposed to alkylating agents, at various concentrations for a further 3 days. Cell viability was determined by the CellTiter 96™ AQ_{ueous} Non-Radioactive Cell Proliferation Assay kit (Promega, Madison, WI) according to manufacturer's instructions. Briefly, 100 μL of cell culture suspension was mixed with 20 μL of MTS/PMS solution and plated in 96-well microtiter plates. Plates were then incubated at 37° for 1 hr in a humidified 5% CO_2 atmosphere, and viable cells were determined by measuring the absorbance at 490 nm on a microplate reader. The quantity of formazan product, and thus the amount of 490 nm absorbance, is directly proportional to the number of living cells in culture. The percentage of surviving cells was calculated from the absorbance at 490 nm and plotted as a function of the drug concentration.

MGMT Activity Assay

Cell extracts were prepared and assayed for MGMT activity as previously described [10, 31].

Northern Blot Analysis

Total RNA from cell cultures was prepared with the Qiagen's RNeasy Total RNA kit as recommended by the manufacturer. RNA (20 μg) was loaded and separated on a 1% agarose gel with formamide, and then transferred to Nitron

Plus membrane (Schleicher & Schuell, Keene, NH). The filters were probed with either [^{32}P]dCTP-labeled full-length MGMT cDNA or a human β-actin cDNA which served as an internal control, by a Random Primers DNA Labeling System (GIBCO BRL). Prehybridization, hybridization, and post-hybridization washes were carried out by standard methods [32]. Filters were exposed to X-ray film at −70° in the presence of a tungsten-intensifying screen.

RESULTS

Generation of High-Titer Producer Cell Lines

A total of 32 GP+E86-MGMT and 20 GP+E86-Neo packaging cell clones were assayed and found to have a titer in the range of 10^5–10^6 CFU/mL of retrovirus-containing medium. Supernatants from clone 6 of GP+E86-MGMT and clone 45 of GP+E86-Neo, which had titers of 4.8×10^6 and 2.4×10^6 CFU/mL, respectively, were used to transduce FDCP-1 cells. Genomic DNA from several of the retrovirus-producing clones was purified and subjected to Southern analysis with MGMT cDNA as a probe. As expected, all GP+E86-MGMT clones showed an integrated 1 kb band, which was not demonstrable with the parent or GP+E86-Neo clones (data not shown). The resulting producer cells not only produced a high titer of retrovirus but also expressed a high level of MGMT activity. As shown in Fig. 2, although the parent GP+E86 cells contained some endogenous MGMT activity, the transfected GP+E86-MGMT cells exhibited 5- to 6-fold higher activity.

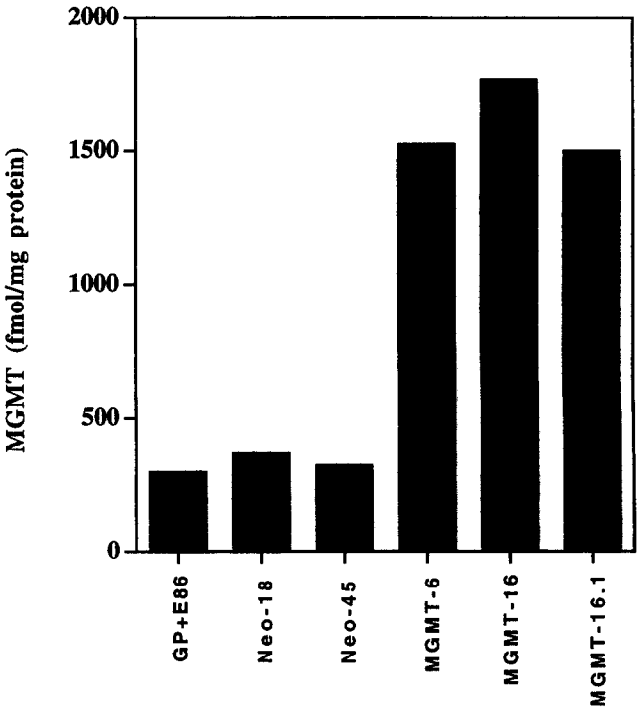

FIG. 2. MGMT activity in retrovirus producer cells. The high-titer retrovirus producer cell lines were generated by transfection of the MGMT-retrovirus vector, pXB/XN-MGMT, into an amphotropic packaging cell line, PA317. The retrovirus-containing supernatant was collected and used to infect an ecotropic packaging cell line, GP+E86 (GP+E86-MGMT). G418-resistant clones were selected and titered on NIH 3T3 cells. The parent vector, which contains only the neomycin-resistant gene, was used as a control (GP+E86-Neo). Cell lysates from the high-titer retrovirus producer cell lines (ranging from 1 to 4 × 10^6 CFU/mL) were collected and assayed for MGMT. The protein activity (ordinate) is given as fmol of methyl moiety transferred to MGMT/mg protein. The results are representative of two independent assays.

Retroviral-Mediated Transfer of mgmt *Gene into FDCP-1 Cells*

FDCP-1 cells, which are MGMT-deficient, were infected in the presence of polybrene with retrovirus-containing supernatants from high-titer retroviral producer cells, either GP+E86-MGMT-6 or GP+E86-Neo-45. Neomycin-resistant cells were selected with G418 and cloned in 96-well plates. A total of 48 MGMT- and 20 Neo-infected clones, named FDCP-MGMT and FDCP-Neo, respectively, were isolated and expanded for determination of MGMT activity. As shown in Table 1, MGMT activity expressed in FDCP-MGMT cells was increased, while activity in the parent FDCP-1 and FDCP-Neo cells remained undetectable. Of the many clones, FDCP-MGMT-617 and FDCP-MGMT-1618 with MGMT activity of 407 and 466 fmol/mg protein, respectively, and FDCP-Neo-453 (as a negative control) were used for the subsequent cytotoxicity studies. The success of this retroviral-mediated transfer of *mgmt* gene into host cells was also confirmed by PCR amplification of the gene using a pair of PCR primers coding for the entire length of the *mgmt* gene (see Materials and Methods) and by Southern blot analysis (data not shown). Northern blot analysis with total RNA isolated from MGMT-transduced FDCP-1 cells and probed with MGMT cDNA probe showed a 4.3 kb major mRNA band and a 1.7 kb minor spliced band (Fig. 3).

Cytotoxicity of the Alkylating Agents to MGMT-Transduced FDCP-1 Cells

The sensitivity of the MGMT-transduced FDCP-1 cells to methylating agents, such as MNNG, MNU, and temozolomide, and to chloroethylating agents, such as CNU and BCNU, was determined. As shown in Fig. 4, the insertion and the functional expression of the human *mgmt* gene in FDCP-1 cells provided a profound protection against the cytotoxicity associated with MNNG, MNU, temozolomide, CNU, and BCNU. Among the five drugs tested, FDCP-MGMT showed the highest protection against MNU (panel B), followed by temozolomide (panel C), and with lesser protection seen with MNNG, CNU, and BCNU (panels A, D, and E) as exemplified in the IC_{50}. The IC_{50} was increased from 5 to >600 μM for MNU; from 4.5 to 165 μM for temozolomide; from 0.5 to 4 μM for MNNG; from 3 to 68 μM for CNU; and from 6 to 24 μM for BCNU, respectively. This experiment suggested that the functional expression of MGMT activity in the FDCP-1 cells could confer a significant resistance to certain alkylating agents,

TABLE 1. MGMT activity in retrovirus-induced FDCP-1 cells

Clones	Specific activity (fmol/mg protein)
FDCP-1	Not detectable
FDCP-Neo	Not detectable
FDCP-MGMT-66	403
FDCP-MGMT-68	530
FDCP-MGMT-616	470
FDCP-MGMT-617	407
FDCP-MGMT-619	490
FDCP-MGMT-1615	538
FDCP-MGMT-1618	466
FDCP-MGMT-1619	414

FDCP-1 cells were transduced with high-titer retrovirus-containing culture medium elaborated by retroviral producer cells, GP + E86-MGMT, in the presence of polybrene. *Neo*^{r} cells were then selected with G418 and cloned in 96-well plates. The individual FDCP-MGMT clones were subsequently isolated and expanded for further characterization. Cell extracts were prepared, and MGMT activity was determined. MGMT specific activity was calculated as fmol/mg of total protein. Clones with MGMT activity higher than 400 fmol/mg of protein are listed here. The results represent the means of two independent assays.

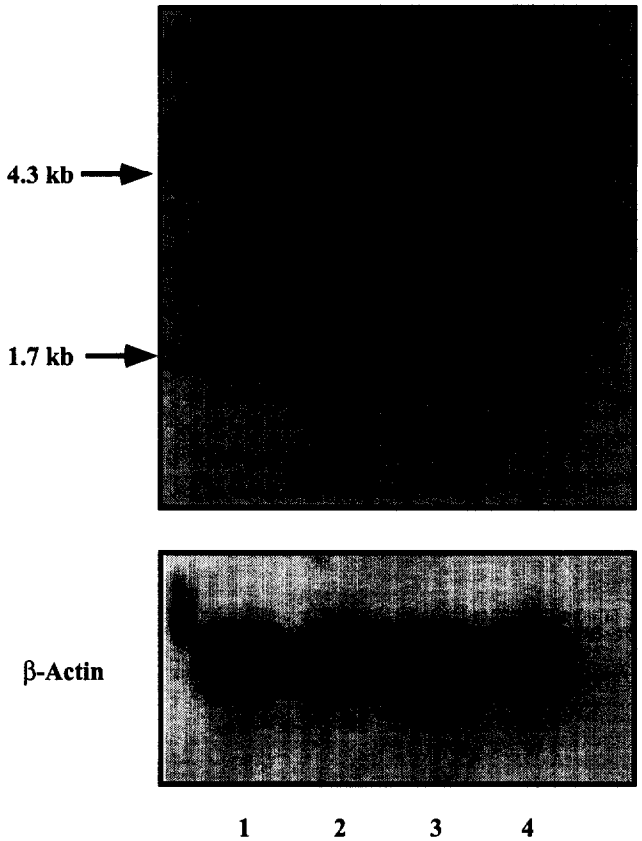

FIG. 3. Expression of MGMT mRNA in MGMT-transduced FDCP-1 cells. Total cellular RNA was isolated from control and MGMT-transduced FDCP-1 cells. Twenty micrograms of RNA was loaded in each lane; the RNA was separated on a 1% formaldehyde-agarose gel and subjected to northern blot analysis with a [^{32}P]dCTP-labeled full-length MGMT cDNA probe. The blot was then washed and reprobed with a β-actin cDNA probe as an internal control. The arrows indicate a 4.3 kb major mRNA band and a 1.7 kb minor spliced mRNA band. Lane 1, control FDCP-1; lanes 2–4, three MGMT-transduced individual FDCP-1 clones.

especially the methylating agents, MNU and temozolomide.

The effects of MNNG, MNU, temozolomide, CNU, and BCNU in the presence and absence of O^6-BG, a potent MGMT inactivator, on the MGMT-transduced FDCP-1 cells were also investigated. As shown in Fig. 4, the depletion of MGMT in transduced resistant FDCP-1 cells by O^6-BG increased their sensitivity to these alkylating agents. In all cases, the IC_{50} with the FDCP-MGMT cells preincubated with 10 μM O^6-BG (a level that inhibits MGMT by >95%) for 1 hr and then treated with different alkylating agents was decreased dramatically; O^6-BG alone had no significant inhibitory effect on either FDCP-1 or FDCP-Neo control cells (data not shown). These results demonstrated that O^6-BG is able to synergize with these alkylating agents in killing bone marrow cells in which the *mgmt* gene had been introduced, suggesting that the protective effect indeed resulted from the functional expression of MGMT and was not due to the possible retroviral insertional activation of some other unknown genes in the host genome.

DISCUSSION

In the clinic, bone marrow toxicity is a dose-limiting factor in the treatment of human malignancies with alkylating drugs. Bone marrow cells appear to be the major target for many of these alkylating agents because of their very low level of the DNA repair protein, MGMT [33–35]. As a consequence, the clinical utility of these alkylating drugs in the therapy of malignant tumors is often compromised by this acute toxicity to bone marrow, such as myelosuppression and later by the appearance of nonlymphocytic leukemia [22].

One approach to solving this problem is to enhance the expression of the DNA repair protein by transducing the *mgmt* gene into human hematopoietic precursor cells. Efficient expression of MGMT and subsequent enrichment following each regimen of chemotherapy would increase bone marrow resistance to the alkylating agent and thereby reduce the risk of chemotherapy-related acute toxicity.

Williams and his colleagues [36, 37] have successfully transferred a methotrexate-cDNA into reconstituting hematopoietic stem cells using a retroviral vector, and found that both primary and secondary recipients transplanted with this recombinant retrovirus showed improved survival and protection from the methotrexate-induced marrow toxicity when compared with controls. Sorrentino *et al.* [38] demonstrated that the retroviral transfer of the human multiple drug resistance gene, *mdr1*, into murine bone marrow cells conferred resistance to taxol. Increased MGMT activity in transgenic mice in which either bacterial *ada* or human *mgmt* gene had been integrated and expressed protected tissues from the toxic effects of certain nitrosoureas and prevented the appearance of nitrosourea-induced tumors [17, 19, 39, 40]. More recently, the human MGMT

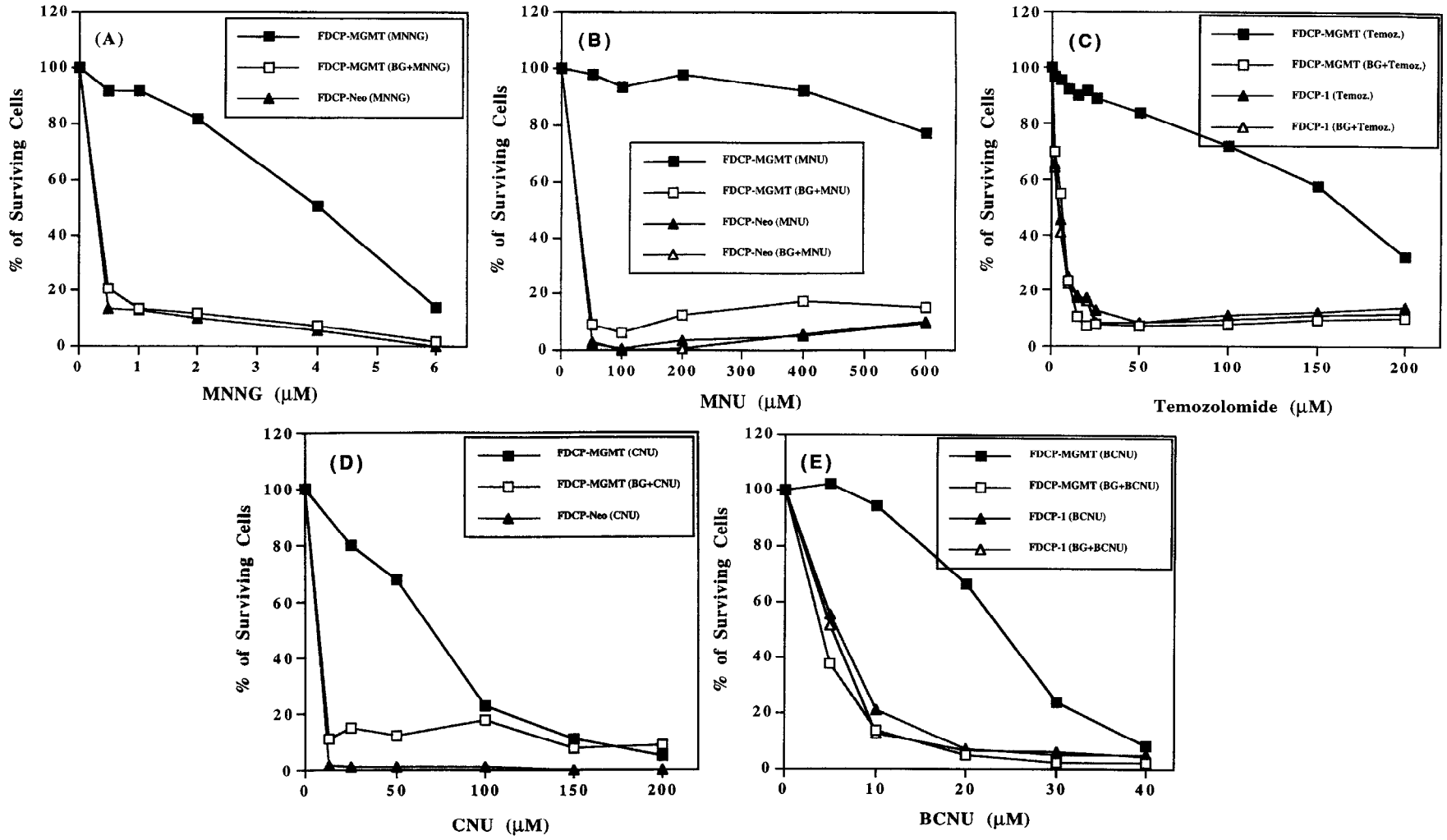

FIG. 4. Effect of O^6-BG and the alkylating agents upon survival of MGMT-transduced FDCP-1 cells. MGMT transduced cells were plated in 6-well plates at a density of 1×10^6 cells/well. Eighteen hours later, the cells were pretreated with or without 10 μM O^6-BG (indicated as BG in the figure) for 1 hr, and then exposed to increasing concentrations of the indicated alkylating agents. The cells were cultured at 37° for 3 days. Cell viability was determined as described in Materials and Methods by MTS/PMS assay. The results represent two independent experiments.

cDNA has been retrovirally transduced and expressed in the murine hematopoietic stem cells of lethally irradiated mice [20, 21]. *In vivo* and *in vitro* experiments with MGMT-transduced bone marrow progenitor cells showed an increased resistance to BCNU. In addition, the transduced mice also expressed a much higher level of MGMT activity in bone marrow, spleen, and thymus.

Because the mature cells of regenerating systems such as intestinal mucosa, skin, and bone marrow all arise from self-renewing stem cells [23], it was important to establish a stem cell line that could express high levels of MGMT activity and with this cell line to determine if the expression of MGMT could provide protection against the cytotoxic effects of certain of the alkylating agents. FDCP-1 cells, an established murine multi-potent hematopoietic stem cell line that was used in this study, have many characteristics that are similar to normal stem cells. For example, under appropriate culture conditions such as in the presence of interleukin-3 and serum factors, the FDCP-1 cells can be induced to undergo multilineage differentiation and development leading to the production of mature cells [24]. Because of these features and of their very low endogenous MGMT, as is apparent in normal bone marrow, FDCP-1 cells appear to be a good model for the *in vitro* study of MGMT protection and cytotoxicity caused by alkylating agents.

In this investigation, we further extended the study of Jelinek *et al.* [23], in which they transfected the FDCP-1 cells by electroporation with the *E. coli ada* and demonstrated that the MGMT-expressing clones were considerably more resistant to the toxic effects of some methylating and chloroethylating agents. We used a retrovirus-mediated transfer system to transduce the human *mgmt* gene into the FDCP-1 cells. With our established retroviral transfer system, we observed a 40–90% transduction efficiency as determined by an *in vitro* high proliferative potential colony-forming cell (HPP-CFC) assay (data not shown). Our results clearly indicated that our retroviral system was able to enhance markedly resistance to the alkylating agents, especially the methylating drugs. The establishment of this high titer retroviral-transfer system provides the appropriate background for the *in vivo* transduction of mouse bone marrow precursor cells with the *mgmt* gene, for the further study of the cytotoxic effects of alkylating drugs on the transduced hematopoietic cells and for providing useful information for future gene therapy applications.

Lesions other than the O^6-alkylguanine adducts must contribute significantly to the sensitivity of FDCP-1 cells to CNU. A likely candidate would be the 1,2-bis-(7-guanyl)-ethane cross-link [41, 42]. The covalent bond between the two guanines from either intrastrand or interstrand G-G cross-links, or any intermediate in their formation would not be substrates for MGMT even though less interstrand cross-links are formed in the presence of MGMT [43]. The greater sensitivity of FDCP-1 cells to the chloroethylating agents, such as CNU and BCNU, than to the methylating agents, such as MNU and temozolomide, and the lesser repair by MGMT may be due to an inability of this protein to repair intrastrand cross-links. This sensitivity can be overcome only partially by the expression of MGMT. Other repair systems might be needed for overcoming the toxic manifestations of the chloroethylating agents upon bone marrow cells. In addition, our results also suggested that we may expect that MGMT would provide a better protection to the bone marrow hematopoietic cells *in vivo* against the hematological toxicity caused by alkylating agents such as DTIC and temozolomide than that by the chloroethylating agent BCNU. Indeed, we have recently transformed murine bone marrow cells with the *mgmt*-bearing retroviral vector and demonstrated expression of this gene using an HPP-CFC assay (Wang G and Bresnick E, unpublished). These transformed cells have been introduced into irradiated mice, and the sensitivity of the recipients to the methylating agents is being determined.

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