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Effect of Extracellular Magnesium on Topoisomerase II Activity and Expression in Human Leukemia HL-60 Cells

Valeria Covacci,[1] Nicodemo Bruzzese,[1] Alessandro Sgambato,[1] Ram Ganapathi,[2] Achille Cittadini,[1] and Federica I. Wolf[1]*

[1]Institute of General Pathology and "Giovanni XXIII" Cancer Research Center, Catholic University of Sacred Heart, 00168 Rome, Italy
[2]Experimental Therapeutics Program, Cancer Center, Cleveland Clinic Foundation, Cleveland, Ohio 44195

Abstract Topoisomerase II (TopoII) is a Mg-dependent enzyme involved in topological modifications of DNA that are crucial to the regulation of cell proliferation and possibly differentiation. To investigate the role of Mg availability in the modulation of TopoII in whole cells, we studied enzyme activity and expression in HL-60 cells grown in the presence of decreasing amounts of extracellular Mg (0.5, 0.03, and 0.01 mM $MgSO_4$). In comparison to cells grown in 0.5 mM Mg, cells grown in 0.03 mM Mg exhibited a decrease in TopoII activity, as evidenced by reduced induction of DNA/TopoII cleavable complexes and apoptosis by etoposide and teniposide. Enzyme activity was restored by the readdition of Mg (0.5 and 1.5 mM) in the incubation medium, confirming that this effect was indeed modulated by extracellular Mg. Restriction of Mg to 0.01 mM was associated with a dramatic decrease in TopoII activity resembling that observed in HL-60 cells differentiated by dimethyl sulfoxide treatment. The restriction of Mg, while decreasing enzyme activity, was found to upregulate TopoII protein expression, determined by Western blot analysis. The increase of TopoII protein levels was correlative with the degree of Mg deprivation. Collectively, these results indicate that extracellular levels of Mg may control availability of intracellular Mg, thus affecting the regulation of TopoII activity/expression and downstream processes of cell proliferation and/or differentiation. J. Cell. Biochem. 78: 325–333, 2000.

Key words: differentiation; proliferation; intracellular Mg pools; Mg-dependent enzymes; human leukemia cells; apoptosis

Topoisomerase II (TopoII) constitutes the major nonhistone component of the nuclear matrix. By repeated break/resealing steps of the double-strand chain, the enzyme resolves some topological problems of the DNA molecule during replication, transcription, recombination, and repair [Anderson and Roberge, 1992]. TopoII is the target for a variety of antineoplastic agents that block the religation reaction forming covalent DNA/TopoII adducts. These "cleavable complexes" interfere with physiologic DNA metabolism and trigger events leading to cell death [Beere et al., 1995]. Several lines of evidence indicate that TopoII plays a role in the commitment of the cell to the differentiation process. In fact, differentiation is accompanied by modification in the activity, protein levels, and phosphorylation of TopoII [Francis et al., 1987; Zwelling et al., 1990; Constantinou et al., 1989; Kaufmann et al., 1991; Aoyama et al., 1999]. Moreover, TopoII cleavage sites have been shown to occur in regions important to the transcription of differentiation-associated genes [Riou et al.,

Abbreviations used: ATP, adenosine triphosphate; CPT, camptothecin; DMSO, dimethyl sulfoxide; FCS, fetal calf serum; Mg, magnesium ions; SDS, sodium dodecyl sulfate; TopoI, topoisomerase I; TopoII, topoisomerase II; VM26, teniposide; VP16, etoposide.

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The first two authors equally contributed to the work.

*Correspondence to: Federica I. Wolf, Institute of General Pathology, School of Medicine, Catholic University–Faculty of Medicine, L.go F. Vito,1 00168 Rome, Italy. E-mail: fwolf@rm.unicatt.it

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1993; Rubin et al., 1991]. Phosphorylation/dephosphorylation of TopoII was suggested to affect DNA by influencing the expression of differentiation- versus proliferation-associated genes [Constantinou et al., 1996]. TopoII inhibitors at noncytotoxic concentrations are also reported to induce the differentiation of various leukemic cell lines [Larsen, 1994]. Finally, TopoII activity is modulated by several protein kinases that are known to influence cell differentiation, for example, casein kinase II [Ritke et al., 1995] and some protein kinase C isoforms [Sahyoun et al., 1986; Corbett et al., 1993; Devalia et al., 1992].

Magnesium ion plays a crucial role in many cell functions. The divalent cation serves both intracellular and extracellular roles, although the relative free concentrations are similar [Cowan, 1995]. Approximately 90% of intracellular Mg is bound to ribosomes, polynucleotides, and adenosine triphosphate (ATP). This bound form has the function to stabilize proteins, nucleic acid, and cell membranes and to participate in the catalysis of several biochemical reactions. The remaining 10% of intracellular Mg is in the ionized form and is quite stable in physiopathologic conditions [Black and Cowan, 1995]. ATP, cytokines, hormones, and prostaglandins can influence Mg homeostasis through receptor-mediated mechanisms that regulate Mg movements across the plasma membrane [Wolf et al., 1996; Keenan et al., 1996]. However, the regulatory role of Mg at the cellular level remains poorly understood, nor is it known how extracellular Mg can influence cell function(s). We have recently demonstrated that human leukemia HL-60 cells induced to differentiate by dimethyl sulfoxide (DMSO) or retinoic acid undergo a substantial decrease of total cell Mg content followed by an intracellular redistribution of Mg that may be important in cell maturation [Wolf et al., 1998; Di Francesco et al., 1998]. In fact, culturing HL-60 cells in Mg-restricted media can influence growth rate and mimic patterns of granulocytic differentiation [Covacci et al., 1998].

To explore the consequences of Mg redistribution previously observed in differentiated HL-60 cells [Wolf et al., 1998], we have studied the effect of extracellular Mg on TopoII activity and expression. TopoII is a potentially ideal target for regulation by intracellular Mg, for at least two major reasons. First, TopoII activity requires Mg as an essential cofactor both for allosteric DNA cleavage and catalytic enzyme-mediated ATPase reaction [Osheroff, 1987]. Second, as mentioned before, because both TopoII expression and intracellular Mg distribution undergo substantial changes during the cell maturation process [Ellis and Zwelling, 1994; Wolf et al., 1998; Di Francesco et al., 1998].

We present data demonstrating that TopoII activity decreases on mild extracellular Mg restriction to 0.03 mM Mg but returns to control levels after reintroduction of Mg (0.5 and 1.5 mM) into the culture medium. Severe extracellular Mg restriction to 0.01 mM causes a more pronounced reduction in TopoII activity similar to that observed in HL-60 cells induced to differentiate by DMSO treatment. Such decrease in TopoII enzyme activity is associated with a significant increase of immunodetectable protein levels, which is correlative with the severity of Mg depletion.

MATERIALS AND METHODS

Cells, Media, and Incubation Condition

Human promyelocytic HL-60 cells were grown as suspension cultures in RPMI 1640 (Bio Wittaker) supplemented with 10% fetal calf serum (FCS) (Biological Industries), at 37°C in 5% CO_2 plus air atmosphere. Mg-restricted medium was prepared with a Mg-free RPMI 1640 (Gibco), supplemented with dialyzed FCS according to Maguire [1988], to which the proper amount of $MgSO_4$ was added to obtain the desired Mg concentration. HL-60 cells referred to as 0.03 mM Mg cells were grown in medium supplemented with 0.03 mM Mg and used the 7th day after subculture; HL-60 referred to as 0.01 mM Mg cells were obtained by subculturing cells adapted to 0.03 mM Mg in 0.01 mM Mg containing medium, and were used on the 4th day of culture. HL-60 cells were induced to differentiate to neutrophil granulocytes by culturing the cells in a medium containing 1.3% DMSO for 7 days.

Determination of Apoptosis

HL-60 cells grown in the presence of varying concentrations of Mg were treated for 5 h with 10 μM etoposide (VP16) (Sigma) and/or 5 μM teniposide (VM26) (Schering Plough). Quantitative determination of apoptosis was performed by fluorescence microscopy on acridine orange–stained cells by scoring for morpholog-

ical features of nuclear pyknosis and chromatin condensation. At least 300 cells were counted for each condition. Double-blind examination was routinely performed. Oligonucleosomal DNA fragmentation was assessed by conventional gel electrophoresis after 5-h treatment with 10 μM VP16, according to Kaufmann [1989] with slight modifications. Briefly, cells were suspended in lysis buffer (10 mM Tris, 10 mM EDTA, 0.5% Triton X, pH 7.4) and incubated at 4°C for 30 min. After ultracentrifugation for 20 min, the supernatant containing small-molecular-weight DNA fragments was transferred to a new tube and was treated with RNAase (400 μg/ml) for 1 h at 37°C followed by ProteinaseK (400 μg/ml) for 1–2 h at 37°C. DNA was then precipitated and loaded onto a 2% agarose gel containing 10 μg/ml ethidium bromide. DNA from 5×10^5 cells was loaded into each lane. Gels were photographed under ultraviolet light Polaroid-type 667 films.

Covalent DNA/Topoisomerases Complex Formation in Intact Cells

Cells were cultured at approximately 1×10^6 cells/ml and labeled overnight with 0.6 μCi/ml [methyl-^{3}H] thymidine (6.70 Ci/mmol, NEN Dupont) and 0.2 μCi/ml [U-^{14}C] leucine (54.0 mCi mmol, Amersham). Cells were then harvested by centrifugation, washed, counted, resuspended in fresh medium, and exposed to 10 μM VP16 or 5 μM camptothecin (CPT) for 1 h. At the end of incubation, each sample was divided into 0.5-ml aliquots and transferred to microfuge tubes. After removal of supernatant, cell pellets were lysed in 1 ml warm (65°C) stop buffer [1.25% sodium dodecyl sulfate (SDS), 5 mM EDTA (pH 8.0), 0.4 mg/ml salmon sperm DNA]. Cell lysates were passed 20 times through a 22-gauge syringe needle, heated for 15 min at 65°C, and buffer supplemented with 112 μl of 1 M KCl, vortexed for 10 s, and incubated on ice for 10 min. The tubes were then centrifuged at 12,000 *g* for 10 min at 4°C. The pellets were heated at 65°C in 1 ml washing buffer (pH 8.0) (10 mM Tris-HCl , 100 mM KCl, 1 mM EDTA, 0.1 mg/ml salmon sperm DNA), incubated on ice, and centrifuged at 10,000 *g* for 5 min at room temperature. The washing procedure was repeated three times. The pellets were finally dissolved in 0.5 ml water at 65°C, transferred into vials containing 4.5 ml of Ultima Gold scintillation cocktail (Packard), and the amount of radioactivity was determined. Data are expressed as ^{3}H-thymidine incorporated into DNA (dpm) normalized for ^{14}C-leucine incorporation as internal control for each sample, or as ^{3}H-DNA/^{14}C-protein ratio, reported as fold increase from basal counts of untreated cells.

Topoisomerase II Decatenating Activity

Decatenating activity of HL-60 cells nuclear extracts was assayed using kinetoplast DNA (kDNA) from *Crithidia fasciculata* (Topogen Inc.) after serial dilution of the same amount of nuclear proteins, following the protocol described in Aoyama et al. [1999].

Topoisomerase Immunoblotting

Cell lysate obtained in RIPA buffer (425 mM NaCl, 50 mM Tris-buffer, 1% NP-40, 0.5% deoxycholate, 0.1% SDS, 10 μM 2β-mercaptoethanol, pH 8.0) were run on a sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-polyacrylamide gel electrophoresis), transferred to a nitrocellulose membrane, and exposed to antibodies specific for TopoI and TopoII (α and β) isoforms. A secondary antibody coupled with peroxidase was used to detect TopoI protein bands by chemiluminescence. Another ^{125}I-labeled secondary antibody was used for recognition and quantification of TopoII protein bands.

Protein were assayed by the Bradford assay using bovine serum albumin as a standard.

Data are presented as mean ± standard error indicated by vertical bars. When vertical bars are not visible, they fall into the symbols. Statistical analyses have been performed by an unpaired Student's *t* test, with significance being considered when $P < 0.05$.

RESULTS AND DISCUSSION

Drug-Induced Apoptosis

To investigate the role of Mg in the regulation of TopoII activity in vivo, we studied the apoptotic response to specific TopoII inhibitors, etoposide (VP16) and teniposide (VM26), in HL-60 cells cultured under Mg restriction or induced to differentiate by treatment with DMSO. The apoptotic response observed under these conditions was assumed as an indirect measure of TopoII activity. Figure 1A shows that DMSO-treated cells were virtually resistant to apoptosis induced by VP16 and VM26.

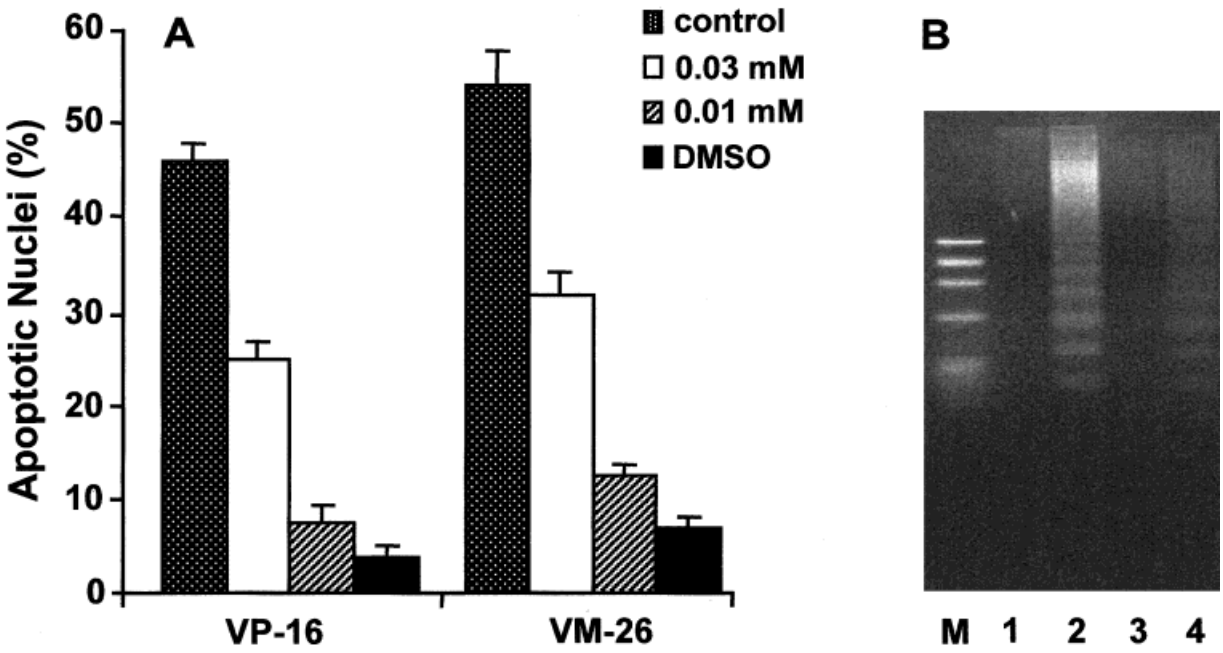

Fig. 1. Drug-induced apoptosis in HL-60 cells. **A:** Morphologic assessment of apoptotic nuclei in control, Mg-deprived, and DMSO-treated HL-60 cells after treatment with VP16 (10 μM) and VM26 (5 μM). Further technical details are given in the Materials and Methods section. Vertical bars indicate the standard errors from the mean of six different experiments. **B:** Agarose gel analysis of DNA oligonucleosomal fragmentation in control and 0.03 mM Mg cells after 5-h incubation with 10 μM VP16. M: molecular weight standard **Lanes 1 and 2:** control cells minus and plus VP16, respectively. **Lanes 3 and 4:** 0.03 mM Mg cells minus and plus VP16.

In cells cultured at an extracellular level of 0.03 or 0.01 mM Mg, apoptosis induced by either drug was reduced by about 40% and 80% as compared to control cells, respectively. In the case of cells cultured in 0.01 mM Mg, resistance to apoptosis was similar to that observed in DMSO-treated cells. Because etoposide-induced cell death requires ongoing DNA synthesis, differences in the cell cycle distribution between control and HL-60 cells grown in the presence of decreasing concentration of Mg could compromise the interpretation of the results. We have previously shown that cells grown under 0.03 mM Mg displayed a cell cycle distribution similar to control cells whereas cell grown under 0.01 mM Mg had a cell cycle distribution similar to DMSO-differentiated cells [Covacci et al., 1998]. Thus, concerning sensitivity to TopoII inhibitors, HL-60 cells grown under 0.03 mM Mg are comparable to control cells, whereas cells grown under 0.01 mM Mg are comparable to DMSO-differentiated HL-60 cells. It is concluded that the differences in drug-induced apoptosis observed in cells grown under restricted Mg concentration are specifically caused by the availability of the cation. Apoptosis was also studied by the typical nucleosomal ladder of DNA fragmentation, which was evaluated after 5 h incubation of control and 0.03 mM Mg cultured cells with 10 μM VP16 (Fig. 1B). The gel obtained after this treatment shows a significant decrease of DNA internucleosomal fragmentation in the 0.03 mM Mg cells (lane 4 versus lane 2), confirming data on apoptosis obtained by morphological evaluation.

To correlate the degree of Mg deficiency to the magnitude of apoptosis, we performed experiments by increasing Mg concentration in the incubation medium during the exposure of HL-60 cells to the drugs. As shown in Figure 2, extracellular Mg did not influence the amount of apoptotic nuclei in cells that had been grown under control conditions. Although the addition of 0.5 or 1.5 mM Mg had no appreciable effect on cells cultured in 0.01 mM Mg, it restored the apoptotic response of cells cultured in 0.03 mM Mg to levels observed in control cells. Taken together, these data suggest that HL-60 cells cultured by restricting extracellular Mg levels exhibit a reduced susceptibility to

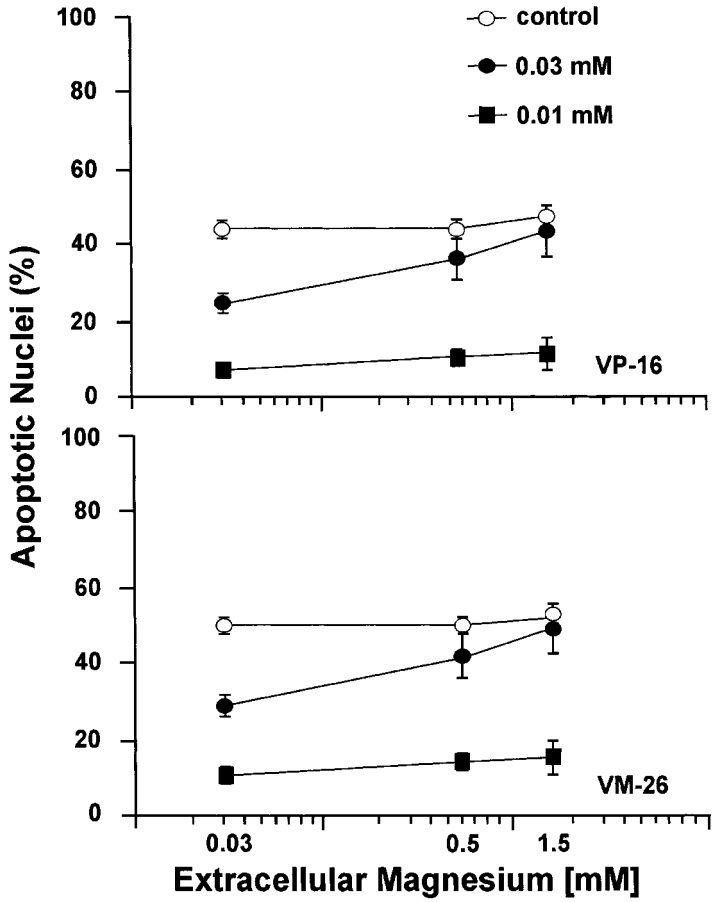

Fig. 2. Effect of extracellular Mg on drug-induced apoptosis in HL-60 cells. Morphologic assessment of apoptotic nuclei in control and Mg-deprived HL-60 cells after 5-h treatment with VP16 (**upper panel**) and VM26 (**lower panel**) in different extracellular Mg concentrations. Data are mean ± standard error of six separate experiments.

apoptosis induced by TopoII inhibitors. Thus, inhibition of TopoII activity by Mg restriction is a key factor of apoptotic response. Furthermore, these data suggest analogies between Mg deficiency and differentiation-induced decrease of TopoII activity. In fact, cells cultured in 0.01 mM Mg exhibit the same resistance to drug-induced apoptosis as observed with DMSO-differentiated cells, which are known to exhibit a significant downregulation of TopoII [Del Bino et al., 1994; Solary et al., 1993].

Before our present study on Mg, the only evidence for TopoII regulation by intracellular cations was obtained in HL-60 cells in which Ca was chelated by treatment with 1,2-bis-(2-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid acetoxymethyl ester. Under those conditions, a decrease of TopoII activity was evidenced by reduced formation of DNA/TopoII cleavable complexes, associated with a hypophosphorylation of the enzyme [Ganapathi et al., 1996; Aoyama et al., 1998].

In principle, Ca and Mg might influence the apoptotic process by activating endonucleases rather than by modulating TopoII [Zhivotovsky et al., 1994; Patel et al., 1994]. This does not appear to be involved in our study, because increasing extracellular Mg from 0.03 to 1.5 mM during treatment with drug did not influence the apoptotic response of cells grown in normal (0.5 mM) Mg medium (Fig. 2). This behavior led us to conclude that Mg deprivation acts upstream of endonuclease-mediated internucleosomal fragmentation.

The observation that extracellular Mg restores apoptosis in 0.03 mM but not in 0.01 mM Mg cells also suggests that severe Mg deprivation may have broader effects similar to cell differentiation induced with DMSO. Indeed, we have previously shown that HL-60 cells treated with DMSO or grown in 0.01 mM cultured HL-60 share similarities that are not confined to the apoptotic response to TopoII inhibitors (e.g., morphologic differentiation, expression of surface antigens, increased oxidative burst) [Covacci et al., 1998].

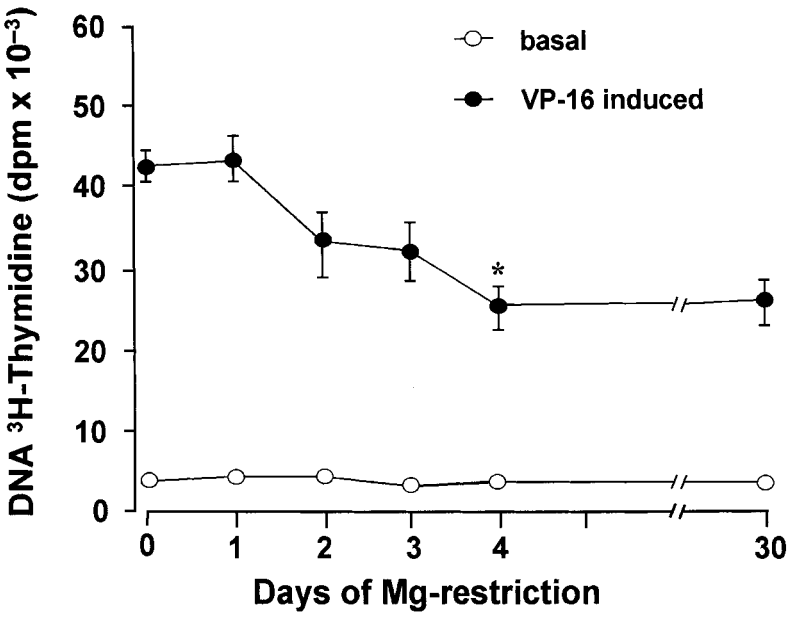

Fig. 3. DNA/TopoII cleavable complex formation of HL-60 cells cultured for different periods in 0.03 mM Mg. Cells were treated with 10 μM VP16 for 1 h and DNA cleavable complexes were determined by the SDS-KCl method (see Materials and Methods for further details). Data are expressed as ^{3}H-thymidine radioactivity (dpm) of precipitated DNA. Data are mean ± standard error of four different experiments. *$P < 0.005$ versus control cells.

DNA/Topoisomerase Complex Formation

To further investigate the role of Mg deprivation on TopoII activity, we evaluated the formation of drug-stabilized DNA/TopoII complexes, which are considered highly indicative of TopoII activity in intact cells. Figure 3 shows the effect of Mg depletion on VP16-stabilized DNA/TopoII cleavable complex formation. After 2 days of culture in 0.03 mM Mg, cells showed reduced formation of TopoII/DNA complexes. This reduction is even more pronounced after 4 days ($P < 0.005$ versus starting value). Prolonged cell culture in Mg-deficient medium did not decrease the formation of DNA/TopoII complexes any further (see also Fig. 3). The progressive reduction of VP16-stabilized DNA/TopoII complex formation strongly suggests that TopoII DNA cleavage activity is inhibited by Mg depletion.

In Figures 1 and 2, we have shown that cells cultured in 0.03 mM Mg are more resistant to VP16- and VM26-induced apoptosis and that such response is reverted by increasing extracellular Mg. To further confirm that this is caused by TopoII activity, we measured DNA/TopoII cleavable complex formation under the same experimental conditions. The results in Figure 4 show that in control cells, DNA/TopoII complexes are not affected by extracellular Mg concentration during VP16 treatment. In contrast, 0.03 mM Mg cells respond to the addition of extracellular Mg by increasing complex formation up to the same levels as observed in control cells. Thus, apoptosis and DNA/TopoII complex formation are dependent on Mg availability, reinforcing the concept that Mg is involved in the regulation of TopoII activity.

In contrast to TopoII, the regulation of TopoI, which acts on a single DNA strand, is independent from Mg [Black and Cowan, 1995]. To further validate our results, we ran similar experiments using CPT instead of VP16 to assess DNA/TopoI cleavable complex formation. In this case, the levels of DNA/TopoI complexes were not modified by extracellular Mg concentration both in control and in 0.03 mM Mg cells (data not shown), further confirming the specificity of the effect of Mg on regulation of TopoII.

TopoII Catalytic Activity

To establish whether the decrease in DNA/TopoII cleavable complex formation was caused by a loss of TopoII catalytic activity, we determined the ability of nuclear extracts from control and Mg depleted cells to decatenate kinetoplast DNA. This assay requires the addition of excess Mg (10 mM) in the reaction mixture [Aoyama et al., 1999]. As shown in Figure 5, no significant difference was observed between the decatenation activity of control and Mg-deprived cells. This result clearly indicates that the ability of TopoII to decatenate DNA is maintained, after cell cul-

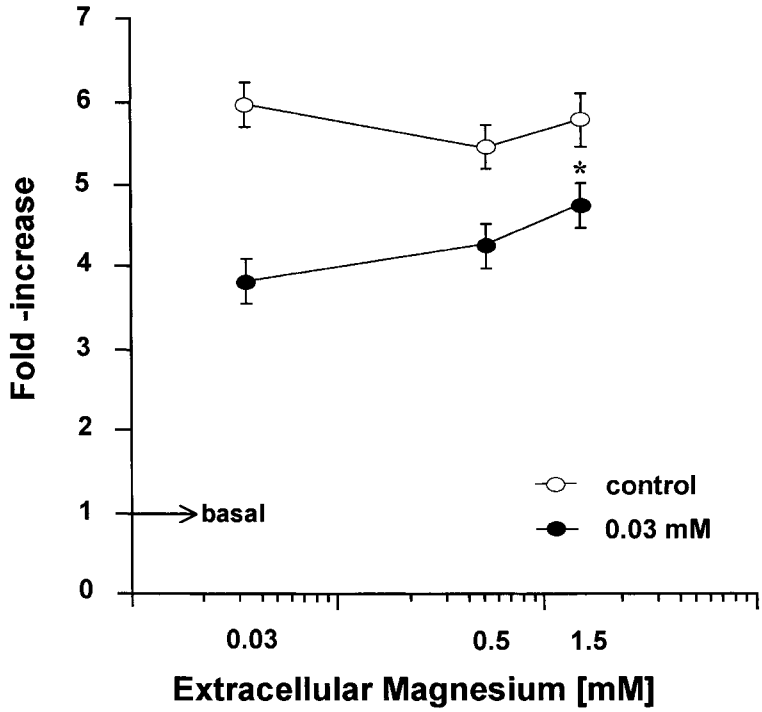

Fig. 4. Effect of extracellular Mg on DNA/TopoII complex formation in control and 0.03 mM Mg HL-60 cells. Cells were treated with 10 μM VP16 for 1 h in the presence of different extracellular Mg concentrations, and DNA cleavable complexes were determined by the SDS-KCl method. Data are expressed as fold-increase over untreated cells and are mean ± standard error of four different experiments. $^*P < 0.05$ versus cells cultured in 0.03 mM Mg.

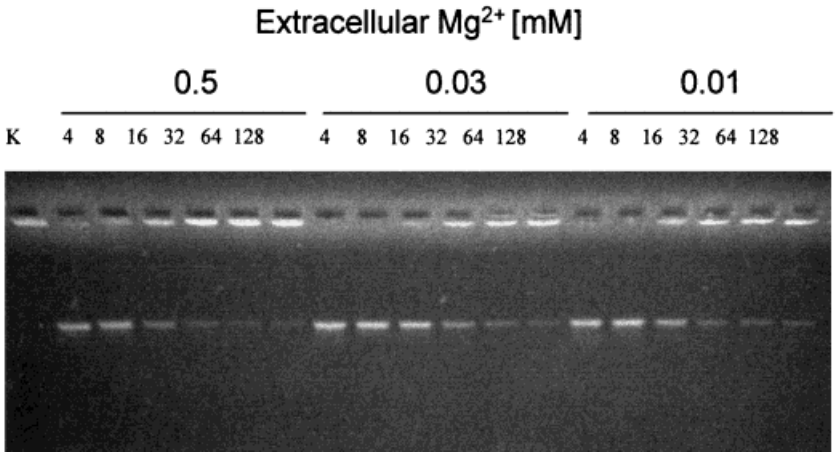

Fig. 5. Decatenating activity of nuclear protein extracts derived from HL-60 cells cultured under Mg restriction (see Materials and Methods section for technical details). Serial dilution (from 4–128) of an equal amount of nuclear proteins was challenged with 200 ng kinetoplast DNA (K), and samples were electrophoresed in agarose gel.

ture in Mg-deficient medium, provided that Mg is present in the assay mixture. Thus, TopoII functions related to apoptosis and to drug-stabilized complex formation are affected by Mg availability, but not the decatenation activity tested in vitro.

TopoII Expression in Cells Cultured Under Mg Restriction

To better understand the mechanism whereby extracellular Mg can influence TopoII activity, we evaluated the enzyme expression by Western blot analysis of protein extracts from HL-60 cells cultured under varying levels of extracellular Mg. As shown in Figure 6, the expression of both TopoII β and α isoforms increased proportionally with the extent of Mg restriction. Densitometric analysis indicated that HL-60 cells cultured in 0.03 and 0.01 mM Mg displayed a twofold and threefold increase of TopoII protein levels, respectively. Conversely, Mg deprivation did not influence the protein levels of TopoI, which is not dependent on Mg for activity. Thus, the correlation between Mg availability and activity but not protein levels of TopoII was observed. The overex-

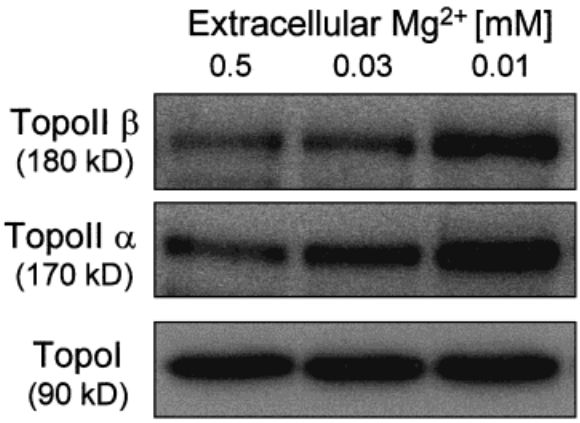

Fig. 6. Immunoblot assay of TopoII α and β isoforms and TopoI expression in HL-60 cells cultured under Mg restriction (from 0.5 to 0.03 and 0.01 mM extracellular Mg). See Materials and Methods section for technical details.

pression of TopoII α and β but not of TopoI previously observed in HL-60 cells induced to differentiate by *all-trans* retinoic acid (RA), was attributed to decreased protein degradation. In the same study, TopoII β was suggested to play a role in the transcription of genes potentially involved in differentiation [Aoyama et al., 1999]. This study also demonstrated that drug-induced apoptosis was shown to decrease significantly in differentiated cells, independent of differences in TopoII protein expression. Our present findings confirm and extend those results, showing that Mg-deprivation can strongly inhibit apoptosis in spite of concomitant increase in the protein levels of TopoII. These data suggest that TopoII protein levels may be increased in an attempt to compensate for reduced Mg availability and consequent loss of enzyme activity. Whether Mg deficiency regulates TopoII levels at transcriptional or posttranscriptional levels remains to be established. Nonetheless, the observation that Mg deprivation has the same effect as RA-induced differentiation lends support to our hypothesis that Mg homeostasis may be a key determinant of differentiation [Covacci et al., 1998].

The mechanism(s) wherein extracellular Mg restriction can influence TopoII activity has been proposed in our previous studies on Mg homeostasis in undifferentiated and neutrophil-like HL-60 cells, where we showed that Mg deprivation triggers a decrease in total cell Mg, leading to redistribution of intracellular Mg from the bound to the free form, which resembles that observed in cells differentiated with DMSO [Covacci et al., 1998; Wolf et al., 1998]. Thus, the decrease of TopoII activity observed in cells grown under Mg deficiency implies that Mg-requiring enzymes may undergo functional inactivation, resulting in modification of related biological processes. The time-dependent decrease in DNA/TopoII complex formation supports this interpretation, as well as the ability of Mg supplementation to restore the formation of such complexes (cf. Figs. 3 and 4). The mechanism(s) whereby Mg influences TopoII activity remain unresolved at the present time. Mg can interact with TopoII directly by promoting DNA cleavage reaction or by affecting enzyme ATPase activity. Alternatively, Mg can act indirectly by influencing the DNA tertiary structure. Mg might also influence TopoII phosphorylation by acting in concert with or antagonistic to calcium ions. Clearly, further studies are needed to delineate the pathophysiologic changes that can mimic these conditions.

In conclusion, this work demonstrates for the first time that in vivo TopoII levels and activity are influenced by manipulating extracellular Mg availability. Based on these findings, we propose that changes in intracellular Mg, induced by physiologic receptor-mediated stimuli, may contribute to the regulation of cell differentiation and programmed death.

ACKNOWLEDGMENTS

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REFERENCES

Anderson HJ, Roberge M. 1992. DNA topoisomerase II: a review of its involvement in chromosome structure, DNA replication, transcription and mitosis. Cell Biol Int Rep 16:717–724.

Aoyama M, Grabowski DR, Dubyak GR, Constantinou AI, Rybicki LA, Bukowski RM, Ganapathi MK, Hickson ID, Ganapathi R. 1998. Attenuation of drug-stimulated topoisomerase II-DNA cleavable complex formation in wild-type HL-60 cells treated with an intracellular calcium buffer is correlated with decreased cytotoxicity and site-specific hypophosphorylation of topoisomerase IIα Biochem J 336:727–733.

Aoyama M, Grabowski DR, Isaacs RJ, Krivacic KA, Rybicki LA, Bukowski RM, Ganapathi MK, Hickson ID, Ganapathi R 1999. Altered expression and activity of topoisomerases during all-trans retinoic acid-induced differentiation of HL-60 cells. Blood 92:2863–2870.

Beere HM, Chresta CM, Alejo-Herberg A, Skladanowski A, Dive C, Larsen AK, Hickman JA. 1995. Investigation of the mechanism of high order chromatin fragmentation observed in drug-induced apoptosis. Mol Pharmacol 47: 986–996.

Black CB, Cowan JA. 1995. Magnesium dependent enzymes in nucleic acid biochemistry. In: Cowan JA, editor. The biological chemistry of magnesium. New York: VCH Publishers. p 137–158.

Constantinou AI, Henning-Chubb C, Huberman E. 1989. Novobiocin- and phorbol-12-myristate-13 acetate-induced differentiation of human leukemia cells associated with a reduction in topoisomerase II activity. Cancer Res 49:1110–1117.

Constantinou AI, Vaughan ATM, Yamasaki H, Kamath N. 1996. Commitment of erythroid differentiation in mouse erythroleukemia cells is controlled by alterations in topoisomerase II α phosphorylation. Cancer Res 56:4192–4199.

Corbett AH, Fernald AW, Osheroff N. 1993. Protein kinase C modulates the catalytic activity of topoisomerase II by enhancing the rate of ATP hydrolysis: evidence for a common mechanism of regulation by phosphorylation. Biochemistry 32:2090–2097.

Covacci V, Bruzzese N, Sgambato A, Di Francesco A, Russo MA, Wolf FI, Cittadini A. 1998. Magnesium restriction induces granulocytic differentiation and expression of $p27^{Kip1}$ in human leukemic HL-60 cells. J Cell Biochem 70:322–331.

Cowan JA. 1995. The biological chemistry of magnesium. New York: VCH Publishers.

Del Bino G, Li X, Traganos F, Darzynkiewicz Z. 1994. Altered susceptibility of differentiating HL-60 cells to apoptosis induced by antitumor drugs. Leukemia 8:281–288.

Devalia V, Thomas NSB, Roberts PJ, Jones HM, Linch DC. 1992. Down-regulation of human protein kinase Cα is associated with terminal neutrophil differentiation. Blood 80:68–76.

Di Francesco A, Desnoyer RW, Covacci V, Wolf FI, Romani A, Cittadini A, Bond M. 1998. Changes in magnesium content and subcellular distribution during retinoic acid induced differentiation of HL-60 cells. Arch Biochem Biophys 360:149–157.

Ellis AL, Zwelling LA. 1994. Time course of phorbol-12-myristate-13-acetate (PMA)-induced down regulation of topoisomerase II in human leukemia cells. Biochem Pharmacol 48:1842–1845.

Francis GE, Berney JJ, North PS. 1987. Evidence for the involvement of DNA topoisomerase II in neutrophil-granulocyte differentiation. Leukemia 1:653–659.

Ganapathi R, Constantinou A, Kamath N, Dubyak G, Gabrowski D, Krivacic K. 1996. Resistance to etoposide in human leukemia HL-60 cells: reduction in drug-induced DNA cleavage associated with hypophosphorylation of topoisomerase II phosphopeptides. Mol Pharmacol 50:243–248.

Kaufmann SH. 1989. Induction of endonucleolytic DNA cleavage in human acute myelogenous leukemia cells by etoposide, camptothecin and other cytotoxic anticancer drugs: a cautionary note. Cancer Res 49:5870–5878.

Kaufmann SH, McLaughlin SJ, Kastan MB, Liu LF, Karp JE, Burke PJ. 1991. Topoisomerase II levels during granulocytic maturation *in vitro* and *in vivo*. Cancer Res 51:3534–3543.

Keenan D, Romani A, Scarpa A. 1996. Regulation of Mg^{2+} homeostasis by insulin in perfused rat liver and isolated hepatocytes. FEBS Lett 395:241–244.

Larsen AK. 1994. Involvement of DNA topoisomerases and DNA topoisomerase inhibitors in the induction of leukemia cell differentiation. Ann Oncol 5:679–688.

Maguire ME. 1988. Magnesium and cell proliferation. Ann NY Acad Sci 551:201–215.

Osheroff N. 1987. Role of the divalent cation in topoisomerase II mediated reactions. Biochemistry 26:6402–6406.

Patel T, Bronk SF, Gores GJ. 1994. Increases of intracellular magnesium promote glycodeoxycholate-induced apoptosis in rat hepatocytes. J Clin Invest 94:2183–2192.

Riou JF, Gabillot M, Riou G. 1993. Analysis of topoisomerase II-mediated DNA cleavage of the c-myc gene during HL-60 differentiation. FEBS Lett 334:369–372.

Ritke MK, Murray NR, Allan WP, Fields AP, Yalowich JC. 1995. Hypophosphorylation of topoisomerase II in etoposide (VP-16)-resistant human leukemia K562 cells associated with reduced levels of βII protein kinase C. Mol Pharmacol 48:798–805.

Rubin E, Kharbanda S, Gunji H, Kufe D. 1991. Activation of the c-jun protooncogene in human myeloid leukemia cells treated with etoposide. Mol Pharmacol 39:697–701.

Sahyoun N, Wolf M, Besterman J, Hsieh TS, Sander M, LeVine H III, Chang KJ, Cuatrecasas P. 1986. Protein kinase C phosphorylates topoisomerase II: topoisomerase activation and its possible role in phorbol ester-induced differentiation of HL-60 cells. Biochemistry 83: 1603–1607.

Solary E, Bertrand R, Kohn KW, Pommier Y. 1993. Differential induction of apoptosis in undifferentiated and differentiated HL-60 cells by topoisomerase I and II inhibitors. Blood 51:1359–1368.

Wolf FI, Di Francesco A, Covacci V, Corda D, Cittadini A. 1996. Regulation of intracellular magnesium in ascites cells: involvement of different regulatory pathways. Arch Biochem Biophys 331:194–200.

Wolf FI, Covacci V, Bruzzese N, Di Francesco A, Sacchetti A, Corda D, Cittadini A. 1998. Differentiation of HL-60 promyelocytic leukemia cells is accompanied by modification of magnesium homeostasis. J Cell Biochem 71: 441–448.

Zhivotovsky B, Wade D, Nicotera P, Orrenius S. 1994. Role of nucleases in apoptosis. Int Arch Allergy Immunol 105:333–338.

Zwelling LA, Hinds M, Chan D, Altschuler E, Mayes J, Zipf TF. 1990. Phorbol ester effects on topoisomerase II activity and gene expression in HL-60 human leukemia cells with different proclivities toward monocytoid differentiation. Cancer Res 50:7116–7122.