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Inhibition of Topoisomerase IIα Activity in CHO K1 Cells by 2-[(Aminopropyl)Amino]Ethanethiol (WR-1065)

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The aminothiol 2-[(aminopropyl)amino]ethanethiol (WR-1065) is the active thiol of the clinically studied radioprotective agent *S*-2-(3-aminopropylamino)ethylphosphorothioic acid (WR-2721). WR-1065 is an effective radiation protector and antimutagenic agent when it is administered 30 min prior to radiation exposure to Chinese hamster ovary K1 cells (i.e., a dose modification factor of 1.4) at a concentration of 4 mM. Under these exposure conditions, topoisomerase (topo) I and IIα activities and associated protein contents were measured in the K1 cell line using the DNA relaxation assay, the P4 unknotting assay, and immunoblotting, respectively. WR-1065 was ineffective in modifying topo I activity, but it did reduce topo IIα activity by an average of 50 percent. The magnitude of topo IIα protein content, however, was not affected by these exposure conditions. Cell cycle effects were monitored by the method of flow cytometry. Exposure of cells to 4 mM WR-1065 for a period of up to 6 h resulted in a buildup of cells in the G2 compartment. However, in contrast to topo II inhibitors used in chemotherapy, WR-1065 is an effective radioprotector agent capable of protecting against both radiation-induced cell lethality and mutagenesis. One of several mechanisms of radiation protection attributed to aminothiol compounds such as WR-1065 has been their ability to affect endogenous enzymatic reactions involved in DNA synthesis, repair, and cell cycle progression. These results are consistent with such a proposed mechanism and demonstrate in particular a modifying effect by 2-[(aminopropyl)-amino]ethanethiol on type II topoisomerase, which is involved in DNA synthesis.

INTRODUCTION

The phosphorothioate WR-2721 has been investigated in clinical trials as an adjuvant for use with radiation (*1,2*) and chemotherapy (*3–5*) to reduce normal tissue toxicity. WR-2721 and its free thiol WR-1065 have also been reported to be effective in protecting against radiation- or chemotherapy-induced mutagenesis (*6–10*) and carcinogenesis (*11–13*). With respect to the antimutagenic effect, these agents significantly reduced the frequency of mutations at the *hprt* locus in cultured mammalian cells and rodent T splenocytes, even when they were administered up to 3 h following exposure of cells to radiation (*6,10*).

The major mechanisms proposed to describe the protective effects of this class of aminothiols have focused on their well-characterized physiochemical properties which include free radical scavenging, hydrogen atom donation, and intracellular auto-oxidation (*14*). The aminothiols have also been implicated in affecting endogenous enzymatic processes in cells. These include DNA synthesis and repair (*15,16*), DNA nuclease activity (*17*), and cell cycle progression (*18,19*) in mammalian cells. As early as 1967, it was proposed that protection by aminothiol compounds is mediated via inherent cellular DNA repair processes (*20*). More recently it was reported that under extracellular conditions the disulfide form of WR-1065 can enhance the topoisomerase-I-mediated unwinding of supercoiled plasmid pIBI30 DNA (*21*). It was also suggested that this result was not unexpected due to the similarity in chemical structure between the disulfide form of WR-1065 and spermine, a polyamine which has been reported to be effective in enhancing the relaxation of DNA supercoils via topoisomerase reactions (*22*).

We have extended studies on the role of aminothiol-mediated effects on DNA-associated enzymes. In particular, we have characterized the effects of WR-1065 (the free thiol form of

WR-2721) exposure on topo I and IIα activities in the CHO K1 cell line (*23*). Effects of WR-1065 on cell cycle progression were also monitored using the method of flow cytometry.

MATERIALS AND METHODS

Cells and Culture Conditions

The CHO K1 cell line was maintained as a stock culture in α–Minimal Essential Medium (Gibco) with 10% fetal calf serum (Biologos) in a humidified atmosphere containing 5% CO_2 and 95% air at 37 °C.

Drug Treatment

WR-1065 used in these studies was supplied by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute. The working solution of WR-1065 was made up in phosphate-buffered saline (PBS) (8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.14 M NaCl, 2.6 mM KCl) at a 1 M concentration and sterilized by filtration immediately before use. Cells were exposed to WR-1065 at a final concentration of 4 mM. At that concentration, WR-1065 is nontoxic and is maximally effective as an antimutagen and a radioprotector (*6–8*).

Survival Studies

Exponentially growing cultures of K1 cells were irradiated with 50-kVp x-rays either in the presence or absence of WR-1065 (4 mM). All experiments were performed in triplicate. Cell survival was determined by plating appropriate numbers of cells to give between 80 and 200 colonies per dish, 6 dishes per experimental point. The D_0 and 95% confidence limits were determined for each survival curve using a computer-fitted, least-squares regression model. Dose modification factors were determined from survival curves by comparing the

corresponding D_0 values obtained for each of the WR-1065 conditions with that of the associated radiation-only controls.

Preparation of Nuclear Extracts

Exponentially growing CHO K1 cells were used in all experiments. Following exposure to radiation and/or WR-1065, treated and control cells in plastic petri dishes were placed on ice, and a volume (equal to that of the culture medium) of cold solution containing 50 mM Tris-HCl (pH 7.5), 25 mM KCl, 2 mM $CaCl_2$, 3 mM $MgCl_2$, and 0.25 M sucrose was added. The cells (about 10^7) were centrifuged and then homogenized. The nuclei were isolated as described by Tandou et al. (*24*) and extracted as described by Champoux and McConaughy (*25*), except that immediately after the washing of cells, a solution comprised of 1 mM phenylmethylsulfonylfluoride, 1 mM benzamidine, 10 μg/ml soybean trypsin inhibitor, 50 μg/ml leupeptin, 1 μg/ml pepstatin, and 20 μg/ml aprotinin was added. The protein content of the nuclear and the cellular extracts was determined by the Bradford method (*26*) and adjusted to 1 mg/ml. Glycerol was added to a final concentration of 30%, and the extracts were stored at −20 °C. These preparations served as the source for topo I and IIα activity, as well as for topo IIα immunoblotting. For the unknotting assay (which detects topo IIα catalytic activity), serial dilutions were made such that the reaction volumes of 20 μl contained a range of 50–800 μg of nuclear extract. For the DNA relaxation assay (which detects topo I activity in the absence of ATP), reaction volumes of 20 μl containing a range of 1–100 μg of nuclear extract were used.

Unknotting Assay for the Determination of Topo IIα Activity

The enzyme sources for this assay were from serial dilutions of nuclear extracts. The substrate used was knotted DNA that had been isolated from tailless capsids of the

bacteriophage P4 Vir1 del10, according to a modification of the methods by Liu et al. (27). Reaction mixtures of 20 μl contained 50 mM Tris-HCl, pH 8.0, 100 mM KCl, 10 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.5 mM EDTA, 40 μg/ml bovine serum albumin (nuclease free), and 1 mM ATP. The reactions were started by the addition of 0.6 μg of knotted DNA and terminated by the addition of 5 μl of a stop solution containing 5% sodium dodecyl sulfate (SDS), 50 mM EDTA, 25% Ficol, and 0.05 mg/ml bromophenol blue. Samples were loaded on 0.8% agarose gels and electrophoresed at 1.5 V/cm for 15 h in Tris/Borate/EDTA (TBE) buffer. Gels were stained in 1 μg/ml ethidium bromide and then destained and photographed over a UV light source. Quantitative determination of topo IIα activity was determined by densitometric measurement of photographic negatives. DNA that remained knotted migrated as a single band to the top of the gels. One unit of unknotting activity is defined as the amount of enzyme that converts 50% of the substrate (knotted DNA) into the reaction product (unknotted DNA).

Relaxation Assay for the Determination of Topo I Activity

The substrate used in this assay was pUC8 plasmid DNA (90% supercoiled). Each reaction volume of 20 μl contained 50 mM Tris-HCl, pH 7.4, 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 mM EDTA, and 30 μg/ml bovine serum albumin (nuclease free). Serial dilutions of the nuclear extracts served as the source of topo I. The reactions were started by the addition of 0.6 μg of the supercoiled plasmid DNA. Following 30-min incubations at 37 °C, the reactions were terminated by the addition of 5 μl of a stop solution containing 50% sucrose, 2% SDS, 0.2 M EDTA, and 0.05% bromophenol blue. Samples were loaded in 0.8% agarose gels, electrophoresed in TBE buffer, stained, and photographed. Densitometric determination of the supercoiled form provided the means for quantitation.

One unit of relaxation is defined as the amount of enzyme that converts 50% of the substrate (supercoiled DNA) into the reaction product (relaxed DNA). The omission of ATP from the assay eliminated the interference of topo IIα activity (*28*).

Immunoblotting for Determining the Topo IIα Protein Levels

Protein content of nuclear and cellular extracts were routinely determined (*26*). Extracts containing 100 μg of protein were analyzed in 8% SDS polyacrylamide gels (*29*). Proteins were transferred electrophoretically to nitrocellulose membranes and incubated with an anti-topo-II antibody recognizing the carboxyl terminal portion of the human topo II. This polypeptide was produced by an expression plasmid p56 z11–1.8 (*30*). The secondary antibody was anti-rabbit IgG. Detection of the bands (corresponding to the 170 kDa form of topo II) was with the peroxidase reaction (Sigma) using 4-chloro-l-naphthol (Sigma) as the color indicator.

Cell-Free Analysis of Topo I and Topo IIα Activity

Relaxation and unknotting assays using purified calf thymus topo I (BRL) or topo IIα purified from HL-525 cells (31) as described by Drake et al. (32), were performed as previously described (33). Reaction mixtures contained WR-1065 at concentrations ranging from 0.4 mM to 40 mM, 0.5 mM Camptothecin (topo I inhibitor, NCI) or 0.3 mM Genistein (topo II inhibitor, ICN Biomedicals). Samples were loaded on 0.8% agarose gels and electrophoresed at 4 V/cm for 5.5 h in TBE buffer. Gels were stained in 1 μg/ml ethidium bromide, destained and photographed over a UV light source.

Flow Cytometry Analysis

The determination of the DNA content of control and WR-1065-treated K1 cells, as well as the evaluation of the effects of WR-1065 treatment on cell cycle progression, was made

using the technique of flow cytometry (FCM). Cells were stained with DAPI (4′,6-diamidino-2-phenylindole) (*34*) in a 0.1% citrate solution according to a method described elsewhere (*35*). FCM patterns were obtained using a PARTEC PAS-III (Particle Analyzing System, Partec AG, Basel, Switzerland) and were analyzed using a computer program obtained from Phoenix Flow Systems, Inc. (San Diego, California, U.S.A.). The coefficient of variation (cv) of the G1 peak obtained using unperturbed cell samples routinely ranged from 1.5 to 2.5 %.

RESULTS

WR-1065 Effects on Radiation Response of K1 Cells

Treatment of K1 cells with 4 mM WR-1065 for 30 min prior to their exposure to 50-kVp x-rays resulted in significant radiation protection, as evidenced by an increase in the survival curve parameter D_0 (i.e., a measure of the terminal slope of the curve) from 2.35 ± 0.15 Gy to 3.35 ± 0.25 Gy (Figure 1). The D_0 values and associated 95% confidence limits were based on a computer-fitted least-squares regression model (*6*). A dose modifying factor (DMF) of 1.4 was determined by taking the ratio of D_0 values from the survival curves describing the response of K1 cells in the presence and absence of WR-1065.

WR-1065 Effects on the Activities of Topoisomerases I and IIα

Topo I and IIα activities were measured in untreated K1 cells along with (a) cells exposed to WR-1065 at a concentration of 4 mM for 30 min, (b) cells exposed to 10 Gy of ionizing radiation, and (c) cells exposed to WR-1065 and ionizing radiation. Presented in Figure 2 are data from an experiment describing topo IIα (panel A) and topo I (panel B) activities extractable from K1 cell lysates. Treatment of K1 cells with 4 mM WR-1065 had

no effect on topo I activity, as seen in panel B. This treatment, however, caused about a 6-fold reduction in topo IIα activity, as determined by densitometric scanning of the photographic negative shown in panel A.

Data from four replicate experiments describing topo I and topo IIα activities from K1 cells are summarized in Table 1. WR-1065 exhibited no effect on topo I activity in K1 cells (all groups compared to controls, $p \geq 0.5$). Topo IIα activity was, however, significantly reduced following exposure to WR-1065, as compared to the untreated control ($p = 0.019$). Following irradiation and exposure to WR-1065, topo IIα activity was somewhat reduced; the level of significance, however, is only suggestive (p value of 0.061).

Since WR-1065 only affected topo IIα activity, topo IIα protein content was also determined for K1 cells exposed to WR-1065. Following exposure to 4 mM WR-1065 for 30 min, exponentially growing K1 cells were lysed, and protein from nuclear extracts were electrophoresed in 8% SDS polyacrylamide gels and transferred to nitrocellulose. Blots were incubated with anti-topo-II antibody which has been previously shown to detect in K-562 cells a single band of 170 kDa corresponding to topo IIα (*36*). Resulting data are presented in Figure 3. The molecular weights presented on the right ordinate are those of topo IIα (MW 170,000) and its proteolytic products. Prestained standards with associated molecular weights are also presented on the left side ordinate. Untreated control cells are represented in lane 1, WR-1065-treated cells are in lane 2, irradiated cells are in lane 3, and irradiated cells treated with WR-1065 are in lane 4. A proteolytic band with a molecular weight of 150 kDa is also evident. Densitometric measurements of protein content (150 kDa and 170 kDa bands) are presented in Table 2 for comparison. No significant differences in topo IIα protein content were observed between any of the experimental groups ($p \geq 0.30$).

Determinations of topo IIα protein contents in cellular lysates of K1 cells were also performed, and data from a representative experiment are presented in Figure 4. Under these conditions, only a single major band of topo IIα was observed for K1 cells, suggesting that the minor band at 150 kDa was due to enhanced proteolytic activity by this cell line.

Examination of the direct effect of WR-1065 on topo I and topo IIα activity is shown in Figure 5. At concentrations up to 40 mM, WR-1065 did not affect the activity of either topo I (panel A) or topo IIα (panel B), as compared to the topo I and II inhibitors Camptothecin and Genistein, respectively. These data suggest that the reduction in topo IIα activity observed in nuclear extracts from WR-1065-treated K1 cells may be due to some indirect effect.

As presented in Figure 6, exposure of K1 cells to 4 mM WR-1065 for up to 6 h gave rise to a buildup of cells in the G2 phase of the cell cycle. The percent of cells in G1 fell from 39 to 21 over this period of time, while the percent of cells in G2 increased from 18 to 27. The percent of cells in S phase was relatively constant, fluctuating only from 43 to 46.

DISCUSSION

Aminothiols such as WR-1065, cysteamine, or glutathione have been reported to be effective in inhibiting DNA synthesis (*15*), strand rejoining (*21*), nuclease activity (*17*), and cell cycle progression (*19*) in mammalian cells. These effects on cellular enzymatic processes have led investigators to propose that one possible mechanism of aminothiol protection involves the modulation of endogenous enzyme processes (*37*).

Radiation protection studies performed on DNA repair-deficient organisms strongly support the suggestion that endogenous enzyme systems are dominant parameters which can

influence the magnitude of aminothiol-mediated protection. While cysteamine was found to be an effective protective agent in wild-type *E. coli*, it was not protective in bacterial strains with defects in the *rec* system (*38,39*). *E. coli* mutants deficient in pol I or UV endonucleases were also not amenable to protection by cysteamine (*39*). Likewise, x-ray repair-deficient *rad*$^-$ mutants of *Saccharomyces cerevisiae* were reported to be unprotectable by cysteamine (*40*). Protection by cysteamine against radiation damage was observed only in diploid wild-type yeast. In addition, WR-1065 was found not to be protective against the lethal effects of radiation on the mutant xrs-5 CHO cell line (*41*).

The aminothiols exhibit close structural similarities to polyamines, which are endogenous polybasic molecules having an affinity for DNA (*42*). The disulfide form (designated WR-33278) of the free thiol WR-1065 has the structure $H_2N-(CH_2)_3-NH-(CH_2)_2-S-S-(CH_2)_2-NH-(CH_2)_3-NH_2$, which is very similar to the structure of spermine (i.e., $H_2N-(CH_2)_3-NH-(CH_2)_4-NH-(CH_2)_3-NH_2$). Spermine, in turn, has been observed to enhance the formation of a stable noncovalent complex between mammalian topo II and DNA (*43*). It also has been reported that WR-2721 and its metabolites WR-1065 and WR-33278 can act as effective substrates and compete with polyamines for uptake into rat lung slices via well characterized polyamine transport processes (*44*). These compounds are also effective substrates for polyamine oxidase activity (*45*). Both aminothiols and polyamines can interact directly with and bind to DNA (*46,47*).

Interest in assessing the effect(s) of the aminothiol WR-1065 on enzymatic activities in K1 cells was prompted in part by these considerations. The focus of this study was limited to an investigation of topo I and IIα because these enzymes are known to be important in DNA synthesis (*48,49*). The conditions used for the preparation of the extracts limit our

observations to the 170 kDa α isozyme of topo II. In contrast to an earlier report in which a cell-free system was used to demonstrate that the disulfide form (WR-33278) of WR-1065 was effective in stimulating the eukaryotic topo I unwinding of negatively supercoiled DNA (*21*), we were unable to observe any effect of WR-1065 at a concentration of 4 mM on topo I activity in K1 cells.

Topo I and IIα activities were unaffected in K1 cells following exposure to ionizing radiation only. However, WR-1065 was effective in inhibiting topo IIα activity. The implications of this phenomenon are at present unclear. Consistent with this inhibition of topo IIα activity, however, was the demonstration that exposure of cells to WR-1065 also induced an arrest of cells in the G2/M phase of the cell cycle (see Figure 6). Topo II poisons such as VP-16, VM-26, and mAMSA are known to induce G2/M phase arrest in cells (*36*). The cytotoxicity of these agents has been attributed to their ability to stabilize the reaction intermediate between topo II and DNA (*50*). WR-1065, in contrast, is an effective radioprotector which is routinely used to enhance cell survival following irradiation or exposure to chemical toxins. The mechanism underlying the ability of WR-1065 to inhibit topo IIα activity may be more analogous to that attributed to spermine and spermidine (*43*). These polyamines are capable of enhancing the formation of a stable noncovalent complex between topo II and DNA, thus facilitating a stimulation of the enzymatic relaxation of DNA. In contrast to the covalent cleavable complexes induced by topo II poisons, the polyamine-induced complex is not associated with the irreversible induction of DNA strand breaks but appears to be part of an endogenous regulatory mechanism involved in DNA synthesis (*43*).

When nuclear lysates were used to measure topo IIα content, a 150-kDa band also was observed. The presence of this band suggests the occurrence of proteolytic activity in the

cellular preparations. The use of rapid cellular lysates, in contrast, demonstrates the presence of only one topo IIα band at 170 kDa (see Figure 4).

The effectiveness of WR-1065 in reducing topo IIα activity in K1 cells and its inability to inhibit enzymatic reactions using purified components in a cell-free system suggest that WR-1065 acts indirectly. One possible mechanism of action may involve the inhibition of protein kinase C-mediated metabolic phosphorylation of topo IIα by WR-1065. (This inhibitory effect has been observed by D. Hallahan, University of Chicago; personal communication.) PKC and other kinases have been shown to phosphorylate topo II metabolically and enhance its catalytic activity (*51–54*). Therefore, agents inhibiting PKC-mediated phosphorylation could reduce the activity of enzymes that serve as substrates for this kinase. This mode of action is consistent with the observed reduction in the catalytic activity of topo IIα in WR-1065-treated K1 cells (determined by the unknotting assay), which is not accompanied by a reduction of topo IIα protein levels (determined by immunoblotting).

Historically, the focus of studies on the mechanism of action of radiation and chemoprotection has centered on the physiochemical properties of the proposed protective agents. In particular, emphasis has been directed toward assessing the abilities of protectors to scavenge free radicals, participate in chemical repair processes via the donation of hydrogen atoms, and the induction of auto-oxidation processes. Another parameter now gathering attention is the ability of these agents to influence endogenous enzymatic processes which, in turn, would influence cellular responses to radiation and chemical insult. The close structural similarity between aminothiols (i.e., WR-1065) and polyamines (i.e., spermidine and spermine) suggests that there may also be a similarity in the mechanism of action exhibited by these agents on cellular enzyme activities involved in DNA synthesis, cell cycle progression and, possibly, repair. In particular, these results extend earlier observations

(*38–40*) of aminothiol interactions with cellular enzymes to now include the ability of WR-1065 to affect the nuclear enzyme topoisomerase IIα.

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TABLE I

The Effects of WR-1065 and Radiation on the Activities of Topo I and IIα in K1 CHO Cells, as Determined by DNA Relaxation and Unknotting Assays, Respectively.[a]

Cell Type	WR-1065	γ-ray	Topo I (Units/μg protein)[b]	Topo IIα (Units/μg protein)[b]
K1	–	–	112 ± 20	59 ± 14
K1	+	–	97 ± 28	26 ± 3[c]
K1	–	+	82 ± 22	53 ± 28
K1	+	+	96 ± 28	36 ± 13[d]

[a]Comparisons made to the corresponding untreated control groups using Student's two-tailed *t* test. Comparisons not significant, $p \geq 0.386$, except as noted.

[b]Mean ± S.D. of four experiments.

[c]Significant difference at $p = 0.019$.

[d]Suggestive difference at $p = 0.061$.

TABLE II

The Effects of WR-1065 and Radiation on the Protein Levels of Topo IIα in K1 CHO Cells, as Determined by Immunoblotting Using an Anti-Topo II Specific Antibody.[a]

Cell Type	WR-1065	γ-ray	100 × Area[b]
K1	–	–	167 ± 55
K1	+	–	179 ± 49
K1	–	+	219 ± 21
K1	+	+	163 ± 39

[a]Comparisons made to the corresponding untreated control groups using Student's two-tailed *t* test. All comparisons not significant, $p \geq 0.300$.

[b]Mean ± S.D. of at least three experiments.

FIGURE LEGENDS

FIG. 1. Survival curves for K1 cells irradiated with 50-kVp x-rays. Cells were either treated with 4 mM WR-1065 (■) or untreated (●). Experimental points represent the mean of three experiments; error bars represent the standard error of the mean. Survival curve parameters were determined by using a computer-fitted least-squares regression model.

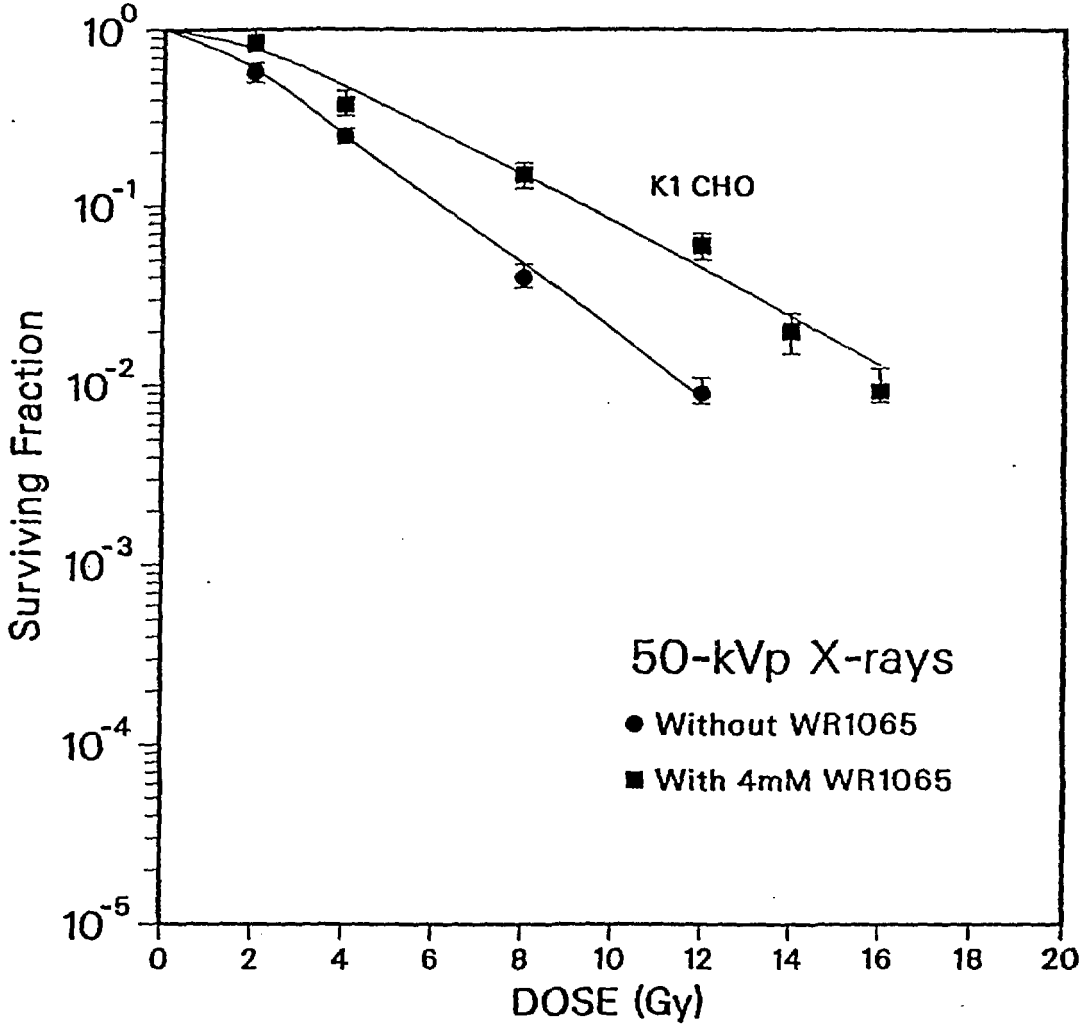
FIG. 2. Topo IIα (panel A) and topo I (panel B) activity in nuclear extracts from untreated and WR-1065-treated K1 cells. Nuclear extracts containing the following amounts of protein were assayed for topo IIα-mediated unknotting and topo-I-mediated relaxing activities, as described in Materials and Methods: panel A, lane 1, 80 ng; lane 2, 40 ng; lane 3, 20 ng; lane 4, 10 ng; lane 5, 5 ng; panel B, lane 1, 100 ng; lane 2, 30 ng; lane 3, 10 ng; lane 4, 3 ng; lane 5, 1 ng; (–), no nuclear extract. This is a representative experiment. Data from four such experiments were used to determine the mean activities.

FIG. 3. Immunoblot analysis of topo IIα levels in nuclear extracts from untreated and WR-1065-treated K1 cells. Logarithmically growing cells were washed twice by centrifugation at 1000 × *g* for 5 min in PBS containing protease inhibitors and extracts. Nuclear proteins were subjected to gel electrophoresis through an 8% SDS-polyacrylamide gel and transferred to nitrocellulose. Blots were incubated with anti-topo II antibody. The molecular weights shown on the right ordinate are those of topo IIα (MW 170,000) and its proteolytic products. Prestained standards with their molecular weights in thousands are shown on the left ordinate. Lane 1, untreated cells; lane 2, WR-1065-treated but unirradiated cells, lane 3; irradiated cells; lane 4, cells irradiated and treated with WR-1065.

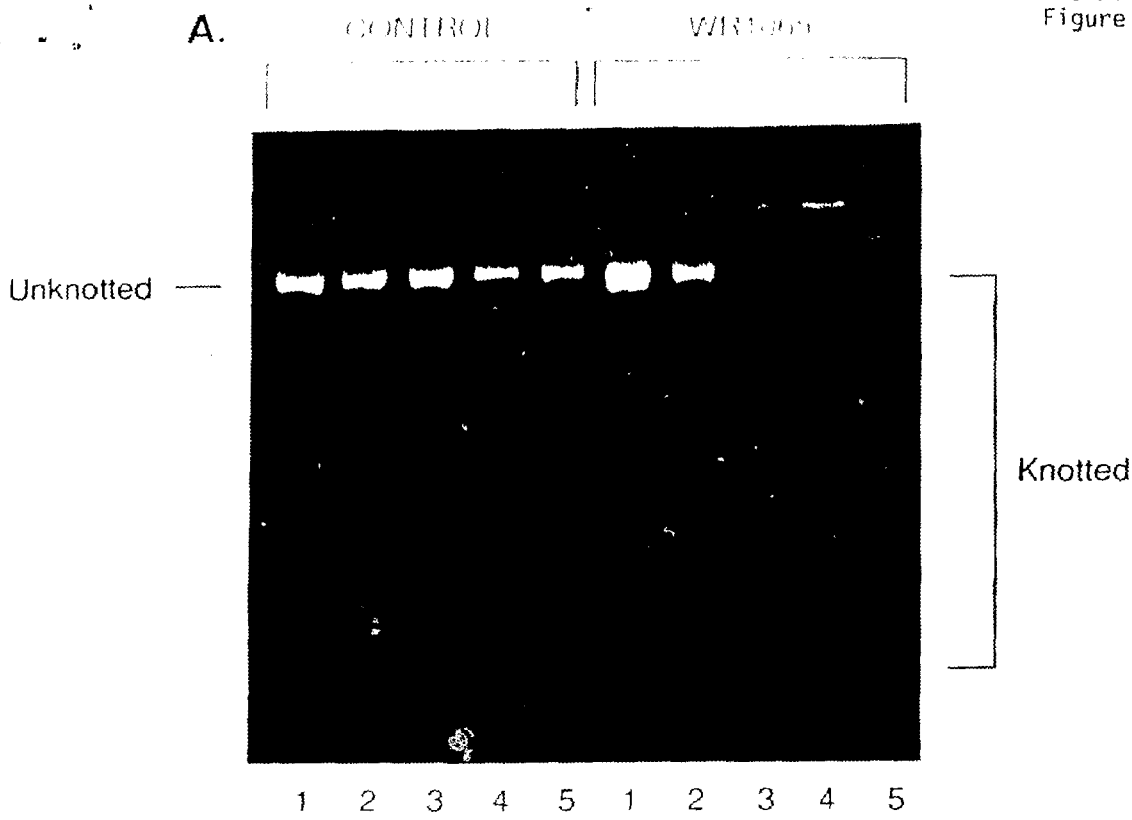
FIG. 4. Immunoblot analysis of topo IIα levels in rapidly lysed cells. Conditions were similar to those described in Figure 3 with the exception that cells were lysed in electrophoresis sample buffer containing 2% SDS by boiling for 2 min.

FIG. 5. Topo I (panel A) and topo IIα (panel B) activity in cell-free extracts. Reaction mixtures were assayed for topo I-mediated relaxation of pUC8 plasmid DNA and topo IIα-mediated unknotting of P4 phage DNA, as described in Materials and Methods: Panel A, lane 1, pUC8 DNA only; lane 2, no drug; lane 3, 0.4 mM WR-1065; lange 4, 4 mM WR-1065; lane 5, 40 mM WR-1065; lane 6, 0.5 mM Camptothecin. Panel B, lane 1, no drug; lane 2, 0.4 mM WR-1065; lane 3, 4 mM WR-1065; lane 4, 40 mM WR-1065; lane 5, 0.3 mM Genistein.

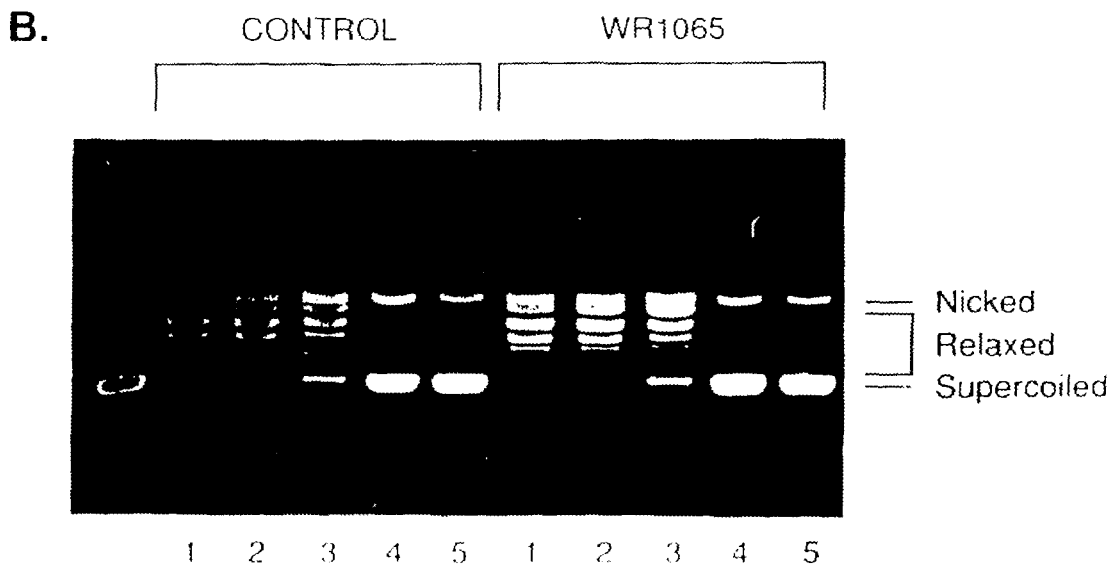
FIG. 6. Typical flow cytometry patterns describing the DNA distribution of K1 cells exposed to 4 mM WR-1065 for 0 min, 30 min, 1–6 h. During the 6 h exposure, the percent of cells in G1 fell from 35 to 21, while the percent of cells in G2 increased from 18 to 27. The percent of cells in S ranged from 43 to 46.

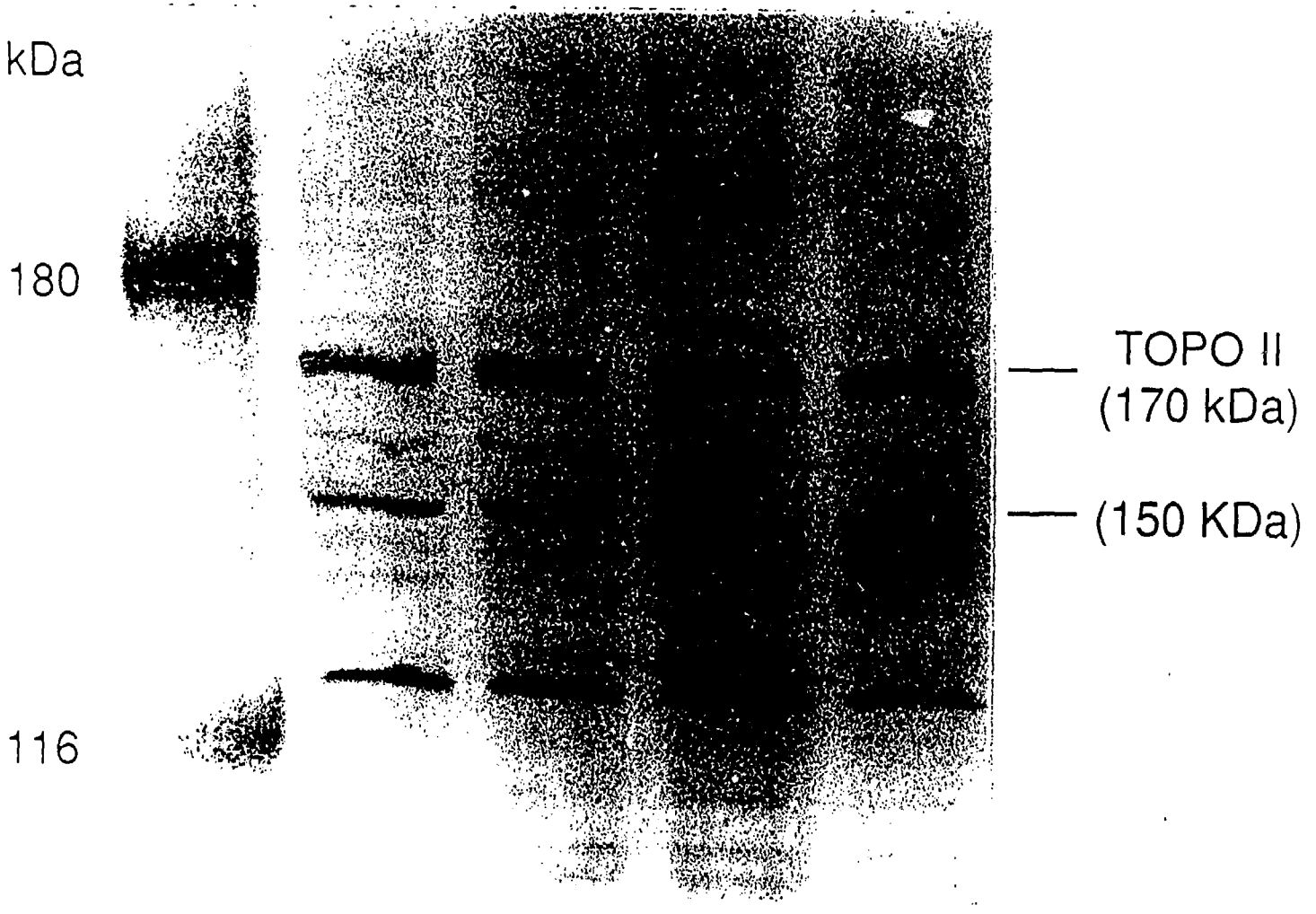

Surviving Fraction
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
K1 CHO
50-kVp X-rays
● Without WR1065
■ With 4mM WR1065
0
2
4
6
8
10
12
14
16
18
20
DOSE (Gy)

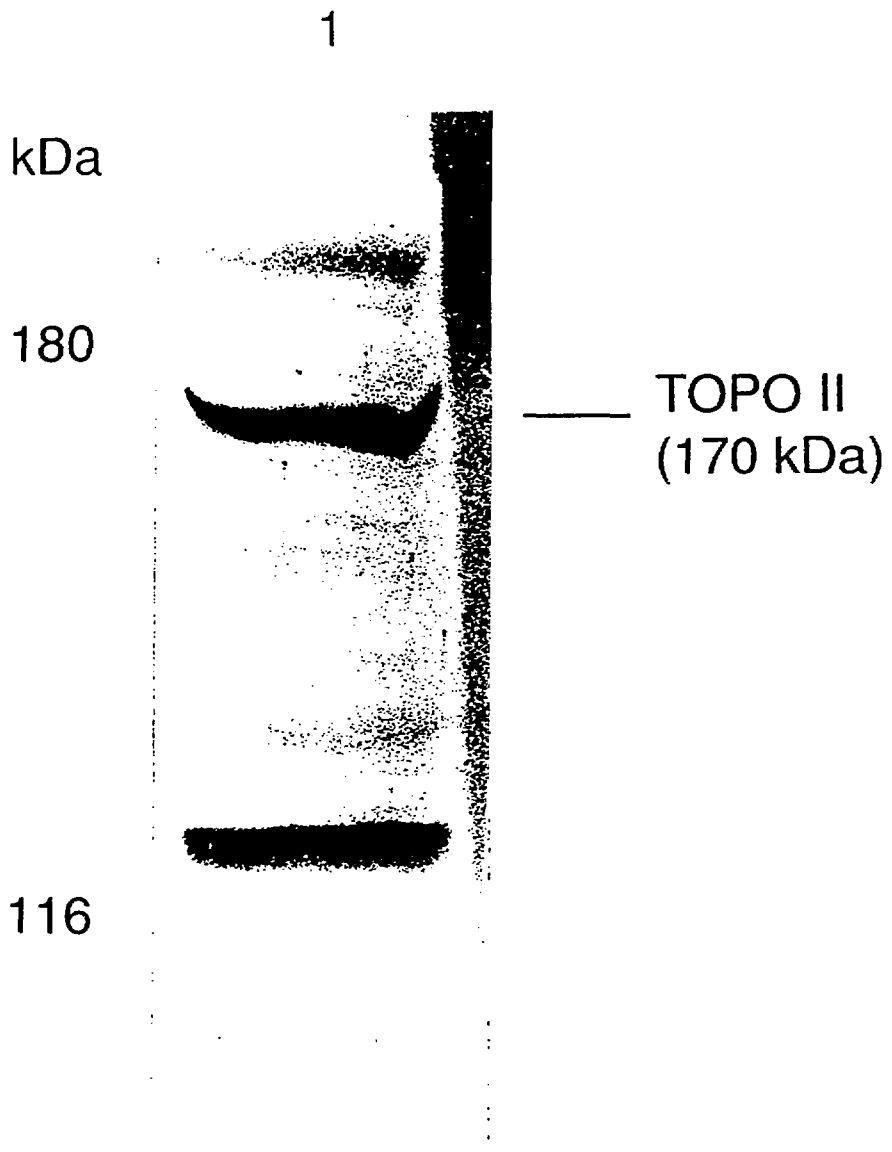
A.

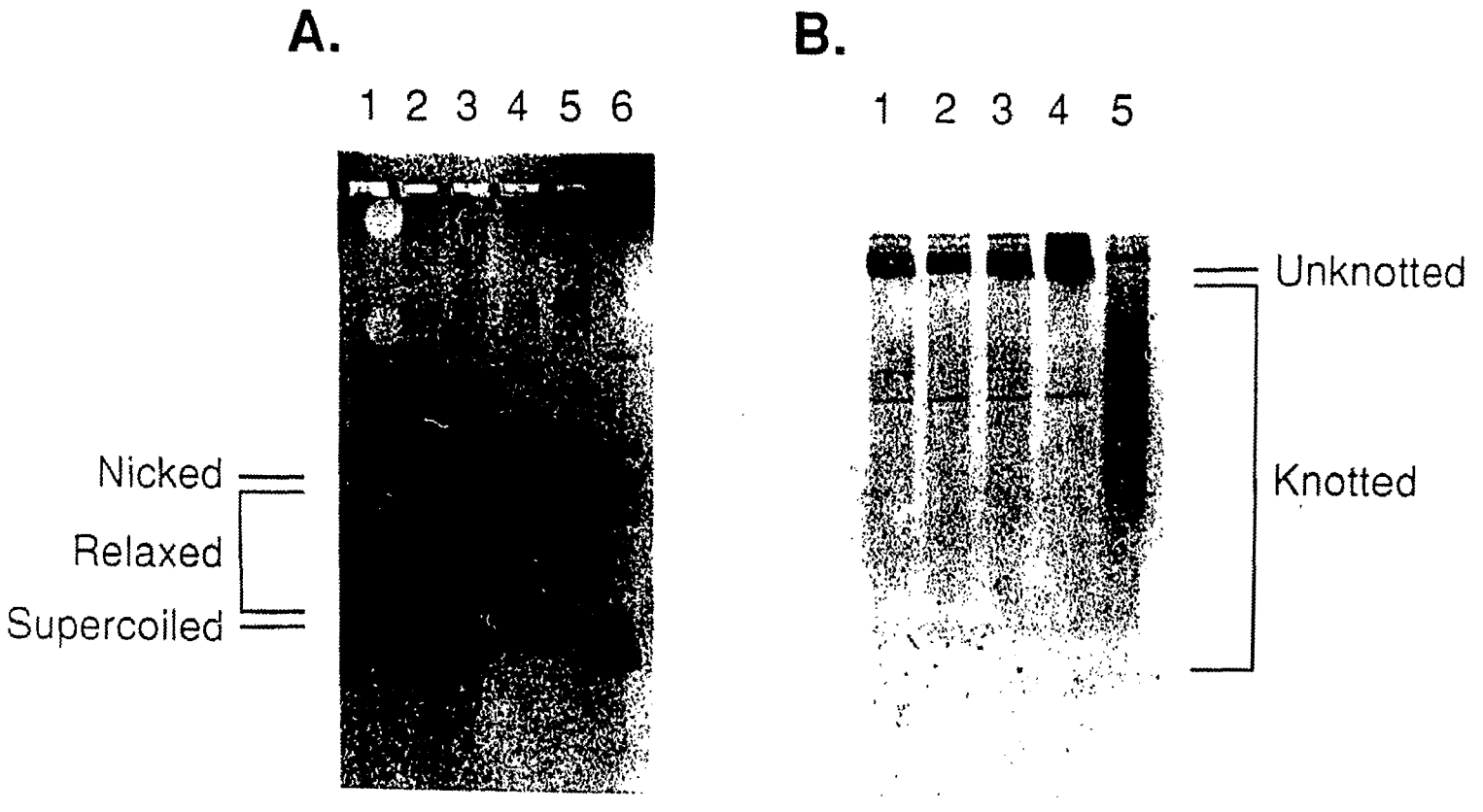

Unknotted
Knotted
1 2 3 4 5 1 2 3 4 5

B.

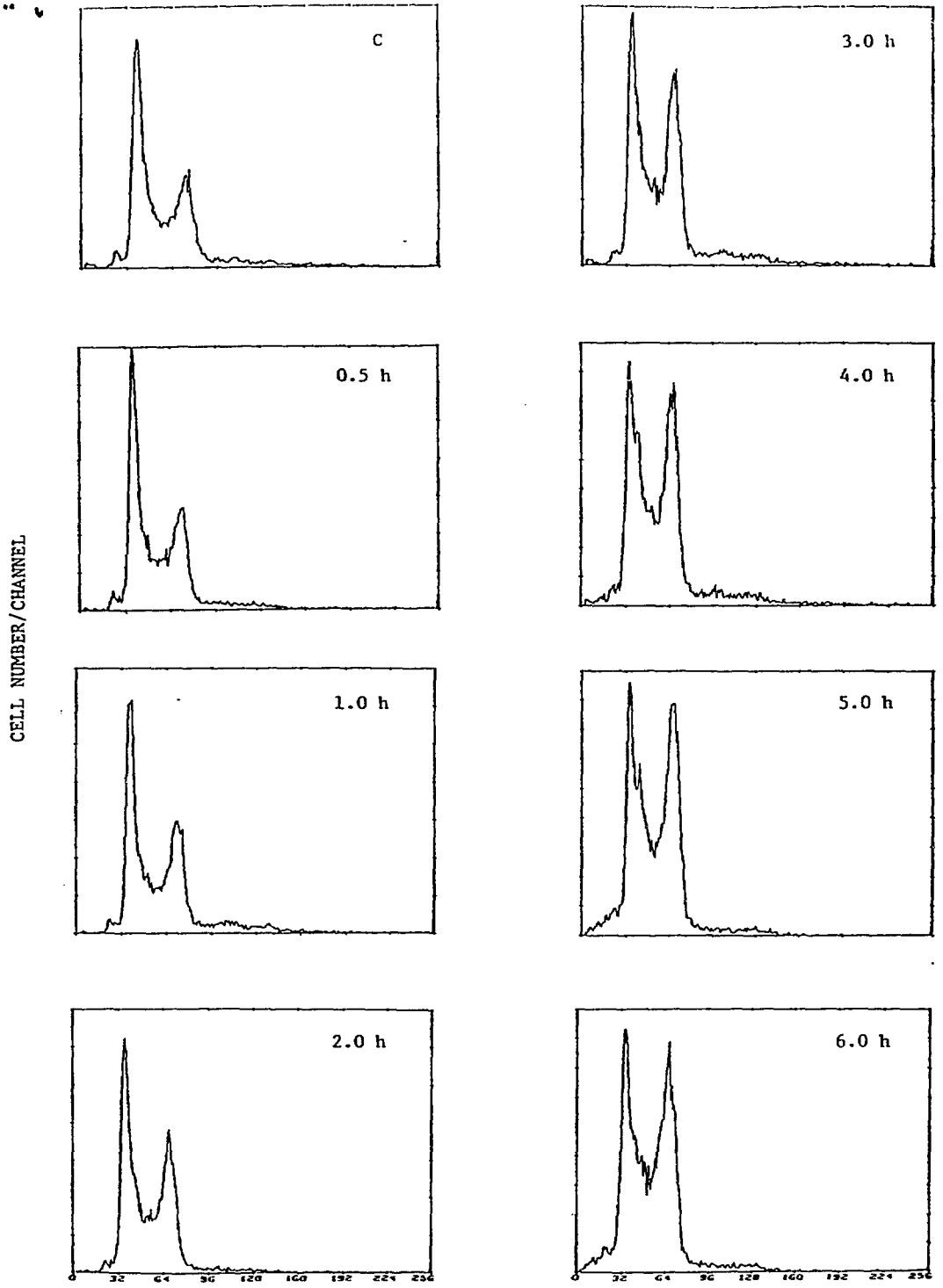

CONTROL
WR1065
Nicked
Relaxed
Supercoiled
1 2 3 4 5 1 2 3 4 5


1
2
3
4
kDa
180
116
TOPO II
(170 kDa)
(150 KDa)


1
kDa
180
116
TOPO II
(170 kDa)



Radiation Research
Grdina, et al.
Figure 5


C
3.0 h
0.5 h
4.0 h
1.0 h
5.0 h
2.0 h
6.0 h
CELL NUMBER/CHANNEL
0 32 64 96 128 160 192 224 256
CHANNEL NUMBER