Published in final edited form as:

J Inorg Biochem. 2011 June ; 105(6): 833–838. doi:10.1016/j.jinorgbio.2011.02.007.

Cadmium is a Catalytic Inhibitor of DNA Topoisomerase II

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Abstract

Cadmium (Cd^{2+}) is a highly toxic and carcinogenic metal that is an environmental and occupational hazard. DNA topoisomerase II is an essential nuclear enzyme and its inhibition can result in formation of genotoxic and recombinogenic DNA double strand breaks. In this study we showed that cadmium chloride strongly inhibited the DNA decatenation activity of human topoisomerase II α in the low micromolar concentration range and that its inhibitory effects were reduced by glutathione. Because the activity of topoisomerase II is strongly inhibited by thiol-reactive compounds this result suggested that cadmium may be binding to critical topoisomerase II cysteine thiols. Cadmium, however, did not stabilize DNA-topoisomerase II covalent complexes, as measured by the lack of formation of DNA double strand breaks. Hence, it is not likely to be a topoisomerase II poison. Consistent with the idea that cadmium cytotoxicity may be modulated by glutathione levels, buthionine sulfoximine pretreatment to decrease glutathione levels resulted in a greatly increased cadmium-induced cytotoxicity in K562 cells. The results of this study suggest that cadmium may exert some of its cell growth inhibitory, and possibly its toxicity and carcinogenicity, by inhibiting topoisomerase II α through reaction with critical cysteine thiols.

Keywords

Cadmium; topoisomerase II; DNA; cytotoxicity; decatenation

1. Introduction

Cadmium is a toxic heavy metal and environmental and occupational exposure to it can result in organ damage and cancer [1,2]. Chronic cadmium poisoning leads to kidney and bone toxicities and prostate and lung cancers [1,2]. Cadmium is retained in the kidney and is nephrotoxic [2]. Cadmium has many effects on cells. These include effects on cellular proliferation, cell cycle progression, cell signaling, DNA replication and repair, differentiation and apoptosis [1,3]. Cadmium can induce cancer in animals and has been classified as a group 1 carcinogen by the International Agency for Research on Cancer

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(IARC) [1,2,4]. It is likely that the carcinogenic effects of cadmium are due to multiple mechanisms, including aberrant gene expression, inhibition of DNA damage repair and oxidative stress [5,6]. Because Cd^{2+} is a redox inactive metal ion, production of free radicals and reactive oxygen species must be occurring through an indirect mechanism [1,5].

We and others have shown that the activity of topoisomerase II α is highly sensitive to thiol-reactive compounds such as acrylamide [7], selenium compounds [8], maleimide [9], cisplatin [10], the organic mercury compound thimerosal [11] and quinones [12-15]. Given the high affinity of Cd^{2+} for thiols [16,17] we decided to investigate whether Cd^{2+} may, in part, be exerting its cytotoxicity by inhibiting DNA topoisomerase II α through reaction with its free cysteine thiol groups. We showed in an earlier mass spectrometric-proteomics study that the topoisomerase II α monomer contains at least 5 free cysteines (amino acids 170, 216, 300, 392, and 405) [10]. Given the critical role of topoisomerase II α in cell division [18-22], this enzyme is a possible target for both the toxic and carcinogenic effects of Cd^{2+} .

Topoisomerase II α is a nuclear enzyme that is critical for cell division and is most highly expressed in cells undergoing division. Topoisomerase II alters DNA topology by catalyzing the passing of an intact DNA double helix through a transient double-stranded break made in a second helix and is critical for relieving torsional stress that occurs during replication and transcription and for daughter strand separation during mitosis [18-22]. Topoisomerase II allows for chromosome separation through its ability to induce temporary double strand breaks in DNA through which DNA is passed. Several widely used anticancer agents such as etoposide, amsacrine, mitoxantrone and doxorubicin are thought to be cytotoxic by virtue of their ability to target topoisomerase II [18-22]. These agents stabilize a covalent topoisomerase II-DNA intermediate (the cleavable complex) and can lead to permanent DNA double strand breaks [18-22]. As has been reviewed the anticancer drugs etoposide, teniposide, doxorubicin and mitoxantrone can induce secondary malignancies by virtue of their ability to target topoisomerase II [20,23]. The catalytic topoisomerase II inhibitors such as dexrazoxane and its analogs are also well known to be leukemogenic [23,24]. In addition, the benzene metabolite 1,4-benzoquinone [14,20,22] and the dietary flavonoid genistein [25] are thought to be leukemogenic through their ability to inhibit topoisomerase II.

In this study we showed that cadmium chloride potently inhibited the decatenation activity of topoisomerase II α and that its inhibitory effects were reduced by glutathione. However, cadmium chloride did not act as a topoisomerase II α poison. The importance of GSH in modulating the activity of cadmium chloride was shown in experiments in which K562 cells were depleted of GSH with BSO.

2. Materials and methods

2.1 Materials

kDNA was obtained from TopoGEN (Columbus, OH) and pBR322 plasmid DNA was obtained from MBI Fermentas (Burlington, Canada). All other chemicals, unless otherwise indicated, were from Sigma-Aldrich (Oakville, Canada). The cell growth inhibition curves were fit to a four-parameter logistic saturation-type equation using SigmaPlot (SyStat, Point Richmond, CA) as described [11,26]. The errors quoted for the IC_{50} values are SEs from the non-linear-least-squares analyses.

2.2 Topoisomerase II α kDNA decatenation inhibition assay

The ability of cadmium chloride to inhibit the catalytic decatenation activity of topoisomerase II α was determined using a spectrofluorometric decatenation assay we described previously [11,12]. Each 20 μL reaction contained 0.5 mM ATP, 50 mM Tris (pH 8.0), 120 mM KCl, 10 mM MgCl_2 , 30 $\mu\text{g/mL}$ bovine serum albumin, 0.2 μM dithiothreitol

from the topoisomerase II α preparation, 40 ng kDNA, GSH or not, and 20 ng of topoisomerase II α protein (the amount that gave approximately 80% decatenation) and cadmium chloride or not, as indicated. Topoisomerase II α was added last to start the reaction. Full length human topoisomerase II α was obtained as we previously described [10]. The reactions were incubated at 37 °C for 20 min, after which time the reactions were terminated by the addition of 12 μ L of 250 mM disodium EDTA. Samples were centrifuged at 8000 g for 15 min at 25 °C to bring down the high molecular weight unreacted catenated kDNA, and 20 μ L of the supernatant was mixed with 180 μ L of 600-fold diluted PicoGreen dye (Molecular Probes, Eugene, OR) in a 96-well plate. Fluorescence, which was proportional to the amount of decatenated kDNA present, was measured in a fluorescence plate reader using an excitation wavelength of 485 nm and an emission wavelength of 520 nm. An agarose gel assay, as we previously described [27], was also used to determine if cadmium chloride inhibited the catalytic decatenation activity of topoisomerase II α and if this inhibition could be reduced by the presence of GSH in the reaction mixture. This assay has the advantage of resolving the different topoisomers of kDNA.

2.3 pBR322 DNA cleavage assay

The drug-induced cleavage of double-stranded closed circular pBR322 DNA to form linear DNA at 37 °C was followed by separating the SDS-treated reaction products using ethidium bromide gel electrophoresis essentially as described, except that all components of the assay mixture were assembled and mixed on ice prior to addition of the drug [11,26]. The 20 μ L cleavage assay reaction mixture contained either 150 ng of topoisomerase II α protein as indicated, 50 ng closed circular plasmid pBR322 plasmid DNA (MBI Fermentas, Burlington, Canada), 0.5 mM ATP in assay buffer (10 mM Tris-HCl, 50 mM KCl, 50 mM NaCl, 0.1 mM EDTA, 5 mM MgCl₂, 2.5 μ g/mL bovine serum albumin, 1.5 μ M dithiothreitol from the topoisomerase II α preparation, 2.5% (v/v) glycerol, pH 8.0) and cadmium chloride or etoposide as indicated. Following the reaction a proteinase K treatment was carried out to digest the covalently bound topoisomerase II from the DNA by treatment with 0.24 mg/mL of proteinase K at 55°C for 30 min as we previously described [28]. The linear pBR322 DNA cleaved by topoisomerase II α was separated by electrophoresis (2 h at 8 V/cm) in a Tris-acetate-EDTA buffer (Tris base (4 mM)/glacial acetic acid (0.11% v/v)/disodium EDTA (2 mM) buffer) ethidium bromide (0.5 μ g/mL) agarose gel (1.2% w/v). The DNA in the gels was imaged by its fluorescence and quantified by densitometry on an Alpha Innotech Fluorochem 8900 imaging system.

2.4 Cell culture and growth inhibition assays

The human leukemia K562 cells, obtained from the American Type Culture Collection (Manassas, VA) and K/VP.5 cells (a 26-fold etoposide-resistant K562-derived sub-line with decreased levels of topoisomerase II α mRNA and protein) [29] were maintained as suspension cultures in α -MEM with 10% fetal calf serum as described [11,26]. For the measurement of growth inhibition, K562 and K/VP.5 cells in exponential growth were harvested and seeded at 6000 cells/well in 96-well plates (100 μ L/well). Twenty-four h later cells were treated with vehicle or various concentrations of cadmium chloride and allowed to grow an additional 72 h. For assessment of the effects of increasing or lowering GSH levels on cadmium chloride cytotoxicity, K562 cells in exponential growth were pretreated in 35 mm culture dishes with either OTC (5 mM) or BSO (100 μ M) or vehicle, respectively, for 24 h. The cells were then seeded (6000 cells/well) into 96-well plates in media that contained either OTC (5 mM) or BSO (100 μ M) or vehicle, respectively. They were then treated with various concentrations of cadmium chloride and allowed to grow an additional 72 h. After treatment cells were assayed with the MTS CellTiter 96 Aqueous One Solution Cell Proliferation assay (Promega, Madison, WI). The spectrophotometric 96-well plate cell growth inhibition assay measures the ability of the cells to enzymatically reduce MTS.

3. Results

3.1 Effects of cadmium chloride on the decatenation activity of topoisomerase II α and its attenuation by GSH

Topoisomerase II α is able to decatenate the highly knotted circular catenated kDNA in an ATP-dependent reaction [18-22]. As shown in lanes 1 and 10 (Fig. 1B), in the absence of topoisomerase II α the extremely high molecular weight catenated kDNA did not move from the origin. Likewise when the decatenation was highly inhibited, as in lanes 8, 9 and 18, most of the kDNA remained at the origin. In the other lanes, where there is only partial or no inhibition of topoisomerase II, there are higher order catenanes (containing 2, 3, 4 or more catenated minicircles) that are present that move slower on the gel than the fully decatenated kDNA. The results in Fig. 1A using the centrifugation assay show that cadmium chloride strongly inhibited the decatenation activity of purified topoisomerase II α with an IC_{50} of $26 \pm 4 \mu\text{M}$. When GSH (500 μM) was included in the reaction mixture the IC_{50} value increased 5.7-fold to $149 \pm 69 \mu\text{M}$. Results from 3 replicate determinations gave an average 4.6 ± 0.6 -fold increase in the IC_{50} value in the presence of added GSH. The gel assay (Fig. 1B) confirmed that cadmium chloride strongly inhibited the decatenation activity of topoisomerase II α and that the inhibition was reduced when GSH (500 μM) was included in the reaction mixture. The changes in fluorescence shown in Fig. 1B of the decatenated kDNA for the Cd^{2+} inhibition of the decatenation reaction in the presence and absence of added GSH bands were quantified using densitometry. A fit to a 4-parameter logistic equation as in Fig. 1A yielded an IC_{50} of 50 μM and 122 μM in the absence and presence of GSH, respectively, which is in reasonable agreement with other decatenation assay results shown in Fig. 1A. The slight difference in IC_{50} values may be due to the fact that the assay of Fig 1A measures all decatenated kDNA (single minicircles and catenanes of 2, 3 or 4 or higher) while the assay of Fig. 1B measures decatenation to single minicircles. The nicked circular (NC) DNA seen in Fig. 1B is typically seen in this decatenation assay and might arise by either of two possible mechanisms. The fully catenated kDNA (which does not move from the origin because of its very large size) may contain nicks from its isolation (like pBR322 DNA typically has) and when it is decatenated by topoisomerase II this nicked DNA can now be seen. Another mechanism might be that topoisomerase II is only causing a partial resealing (one strand only) of the DNA double strand break during the double strand passage event.

3.2 Inhibition of topoisomerase II α activity by cadmium chloride was not accompanied by stabilization of the covalent topoisomerase II α -DNA cleavable complex

Topoisomerase II-cleaved DNA complexes produced by anticancer drugs may be trapped by rapidly denaturing the complexed enzyme with SDS [11,26,28,30]. Typically, high drug concentrations are used in this assay in order to be able to trap and detect the normally transient covalent enzyme intermediate [18-22,30]. It should be noted that this assay is not directly measuring a covalent interaction of topoisomerase II and pBR322 DNA, but it is only measuring it indirectly through formation of double strand breaks in the DNA. DNA cleavage assay experiments were carried out using etoposide as a positive control to see whether cadmium chloride stabilized the cleavable complex. As shown in Fig. 2, the addition of 100 μM etoposide in the reaction mixture induced formation of linear pBR322 DNA (lane 1), an indication of DNA double strand breaks. Linear DNA was identified by comparison with linear pBR322 DNA produced by action of the restriction enzyme *HindIII* acting on a single site on pBR322 DNA (data not shown). The bands were quantified by densitometry and relative to the no drug control of lane 3, the integrated fluorescence for the linear DNA band for 0.1 mM etoposide, 0.1 mM Cd^{2+} and 1 mM Cd^{2+} is 1562, 138 and 335. The 0.1 mM Cd^{2+} and 1 mM Cd^{2+} values were 11- and 5-fold lower than for etoposide. Given the errors in quantitating a gel of this type the 0.1 mM Cd^{2+} treatment clearly did not

produce linear DNA, and the 1 mM Cd^{2+} treatment only marginally increased linear DNA over the control values, at least compared to etoposide. Thus, the addition of either 0.1 or 1 mM cadmium chloride to the reaction mixture induced little or no detectable formation of cleaved linear pBR322 DNA (Fig. 2).

3.3 Comparison of the effect of cadmium chloride on the growth of a K562 cell line with a K/VP.5 cell line with a reduced level of topoisomerase II α

The K/VP.5 cell line contains one-fifth the amount of topoisomerase II α compared to the parent K562 cell line [31], and can be used to test whether a compound is a topoisomerase II poison [10]. Fewer DNA strand breaks will be produced in cells containing less topoisomerase II, and thus topoisomerase II poisons will be less inhibitory in the K/VP.5 cell line. As shown in Fig. 3, the IC_{50} for cadmium chloride growth inhibition was 19.0 ± 0.9 and 12.2 ± 0.4 μM , respectively for K562 and K/VP.5 cells. Thus, even though cadmium chloride inhibited the catalytic activity of topoisomerase II α (Fig. 1), the lack of cross resistance in the K/VP.5 cell line suggests that it did not act as a topoisomerase II poison in a cellular context. This result is also consistent with the inability of cadmium chloride to stabilize the DNA-topoisomerase II α cleavable complex and induce formation of linear DNA (Fig. 2). For comparison we have previously shown that etoposide has an IC_{50} for growth inhibition of 0.07 μM and 1.8 μM for K562 and K/VP.5 cells, respectively [29].

3.4 Reducing intracellular GSH levels increases cadmium chloride cytotoxicity in K562 cells

Because Cd^{2+} has a high affinity for GSH [17,32] we also investigated whether supplementation or depletion of intracellular GSH levels affected the cytotoxicity of cadmium chloride. As we reported previously [11,33], GSH levels in K562 cells were increased (by treatment with OTC) or decreased (by treatment with BSO). OTC is a non-thiol precursor of cysteine which is converted by intracellular 5-oxoprolinase to cysteine, a precursor for the biosynthesis of GSH [34]. GSH levels were decreased in K562 cells by a 24 h preincubation with 100 μM BSO, an inhibitor of the rate limiting GSH-synthesizing enzyme γ -glutamylcysteine synthetase [34]. Using a fluorescent Thioglo-1 thiol assay we previously described [33], cellular GSH levels in BSO-treated K562 cells were reduced to one-sixth of their normal value. We also previously showed that cellular thiols in K562 cells increased 1.9-fold following OTC treatment [11,33]. Because the Thioglo-1 assay measures non-protein thiols, the OTC-induced increase in thiol levels likely reflects an increase in both cysteine and GSH levels. The OTC or BSO pretreatments were followed by a 72 h-exposure to various concentrations of cadmium chloride, after which cell growth was measured with the MTS assay. As shown in Fig. 4, cadmium chloride inhibited cell growth with an IC_{50} value of 18 ± 4 μM for untreated cells. For the OTC-treated cells the IC_{50} value increased slightly (1.3-fold to 23 ± 4 μM). For the GSH-depleted BSO-treated cells the IC_{50} value was reduced 23-fold to 0.77 ± 0.12 μM .

3.5 Effect of pretreating cadmium chloride with either GSH or 2-mercaptoethanol on its cytotoxicity towards K562 cells

In a separate experiment a stock solution of 40 mM cadmium chloride was pretreated with either fresh 160 mM GSH (pH 7.5) or 2-mercaptoethanol for 1 h at 37 °C in water. Microliter amounts of this thiol-treated stock solution were then added to the K562 cell suspension. Thus, in these experiments, the thiol concentration in the medium was always 4-fold higher than the cadmium chloride concentration. In this experiment the IC_{50} value for cadmium chloride was 19 ± 1 μM . The GSH and 2-mercaptoethanol treatments only slightly decreased the IC_{50} values to 17 ± 1 μM and to 18 ± 1 μM , respectively (supporting information, Fig. S1).

3.6 Effect of a 1 h cadmium chloride treatment on K562 cells that were pretreated with GSH

In this experiment K562 cells in PBS (pH 7.4, NaCl (155 mM), KH_2PO_4 (1 mM), Na_2HPO_4 (3 mM)) in the absence or presence of GSH (2 mM) were treated with cadmium chloride at 37 °C for 1 h. The plate was centrifuged and the PBS was removed and replaced with culture media containing no added GSH and the cells were allowed to grow for a further 144 h. The IC_{50} value for the untreated cells was $14 \pm 3 \mu\text{M}$. The IC_{50} value increased 11-fold to $155 \pm 45 \mu\text{M}$ for the cells that were pretreated with GSH (supporting information, Fig. S2). The IC_{50} value of $14 \mu\text{M}$ for this 1 h cadmium chloride treatment compares to the IC_{50} value of $19 \mu\text{M}$ for a 72 h continuous treatment (Fig. 3). In contrast to the results from the previous experiment, pretreatment of cells with GSH resulted in a dramatic decrease in cadmium chloride growth inhibitory effects.

4. Discussion

Since topoisomerase II has been shown to have high sensitivity to thiol-reactive compounds [7-9,11-15], we investigated whether cadmium chloride inhibited topoisomerase II α through reaction with its free cysteine groups. Given the critical role of topoisomerase II α in cell division [18-22], this enzyme is a possible target for the toxic, genotoxic and carcinogenic effects of cadmium. In this study, we showed that cadmium chloride was a potent inhibitor of the catalytic activity of topoisomerase II α (Fig. 1) and that this inhibition of topoisomerase II α was strongly reduced by the addition of GSH to the reaction mixture. These results suggests that inhibition occurs through binding of Cd^{2+} to critical free cysteine thiols on topoisomerase II α . Topoisomerase II α has at least 5 free cysteines per monomer [10]. It is well known that Cd^{2+} has a high affinity for thiols such as GSH [16,17]. Under physiological conditions it was estimated that, at a total Cd^{2+} concentration of $6 \mu\text{M}$ and a GSH concentration of 0.82 mM (which approximates cellular GSH concentrations), 95 % of the Cd^{2+} would be bound to GSH [32]. Thus, under conditions that we treated K562 cells (Fig. 3), a maximum of only $0.3 \mu\text{M}$ out of a starting concentration of $6 \mu\text{M}$ Cd^{2+} would not be bound to GSH. Hence, Cd^{2+} may be a potent inhibitor of cellular topoisomerase II and/or other proteins exerting its cytotoxic actions through critical cysteine thiol group interactions. It may be that formation of even a transient Cd^{2+} adduct with a critical cellular component during an essential biotransformation event may be enough to induce irreversible cell damage. Also, under conditions similar to those of this study, Cd^{2+} has also been shown to have an association binding constant for ATP about 7-fold higher than for Mg^{2+} [35]. However, given that the Mg^{2+} in the decatenation assay (Section 2.2) is present at 10 mM, the fraction of ATP that would be bound to Cd^{2+} compared to that bound to Mg^{2+} would be very small. Thus, Cd^{2+} binding to ATP would not be expected to contribute significantly to its ability to inhibit the catalytic activity of topoisomerase II α . It is well noted that even though Cd^{2+} is a strong inhibitor of K562 cell growth (Fig. 3), this inhibition cannot be assumed to necessarily be related to the inhibitory effects of Cd^{2+} on topoisomerase II.

Depletion of GSH to one-sixth of their normal levels in K562 cells by BSO treatment resulted in a 23-fold decrease in the cadmium chloride IC_{50} value towards K562 cells (Fig. 4) indicating the importance of cellular GSH in protecting cells. These results are similar to what we observed with thimerosal-treated K562 cells [11]. These results are also consistent with mouse studies in which it was shown that BSO pretreatment increased Cd^{2+} toxicity [36]. It is also noted that depletion of cellular GSH compromises a major antioxidant system of the cell. Thus, even though Cd^{2+} is not itself redox active, it does indirectly induce oxidative stress [1,5,6]. GSH depletion and loss of its antioxidant effects would be expected to contribute to the cytotoxicity of Cd^{2+} . Increasing cellular thiol levels by 1.9-fold following OTC treatment increased the cadmium chloride IC_{50} values only 1.3-fold (Fig. 4). Thus, it would appear that increasing GSH levels does not greatly increase protection because endogenous GSH levels are sufficient to protect the cell..

Experiments were also carried out in which cadmium chloride stock solutions were pretreated with a 4-fold excess of either GSH or 2-mercaptoethanol which was then added to K562 cells (Fig. S1). In contrast to the ability of GSH to protect against cadmium chloride-induced inhibition of topoisomerase II α decatenation activity (Fig. 1), the protection offered by thiol pretreatment of cadmium chloride in the cellular assay was not large (approximately 2-fold increase in the IC_{50} value).

It has been shown that Caco-2 cells have a high affinity, but low capacity, system for transporting the Cd²⁺-GSH complex into cells [32]. Our results showing little effect of either GSH or 2-mercaptoethanol on cadmium chloride IC_{50} values are consistent with a similar low capacity Cd²⁺-GSH transport system also operating in K562 cells. It should also be pointed out that the topoisomerase II α enzyme inhibition assay was carried out for 20 min, while the cellular assays were carried out over 72 h. The much longer timescale for the cellular assay would favor Cd²⁺ eventually entering the cell and exerting its cytotoxic effects. Thus, it would appear that binding of Cd²⁺ to GSH in the enzyme assay protects the enzyme by lowering the effective Cd²⁺ concentration, but binding of Cd²⁺ to GSH in the extracellular environment does not greatly affect the ability of Cd²⁺ to enter cells and exert its cytotoxicity.

Another contributor to the cytotoxicity of Cd²⁺ in these cellular experiments may be due to the lability of GSH-bound Cd²⁺ which could allow Cd²⁺ to rapidly exchange with and target other critical protein and non-protein thiols. GSH has been shown in NMR experiments to undergo rapid exchange on the millisecond timescale with a Cd²⁺-nitrilotriacetic acid complex [16,17]. The results of the experiment shown in Fig. S1 in which cadmium chloride stock solutions were pretreated with either GSH or 2-mercaptoethanol were somewhat unanticipated. However, these results may be explained by the ability of Cd²⁺ to undergo rapid exchange with its ligands [16,17]. Typically divalent metal ions such as Cd²⁺ undergo rapid solvent and ligand exchange reactions [37]. It is possible that the preformed Cd²⁺-GSH adducts in the experiment described in Fig. S1 exchanged with critical protein cysteine thiols, either in the cell or on the cell surface, and thus contributed to its cytotoxicity. The results of Fig. S2 that show that a 1 h Cd²⁺ treatment is strongly growth inhibitory suggests that Cd²⁺ rapidly entered the cells. This result is consistent with the rapid entry of Cd²⁺ within cells [32]. The fact that the IC_{50} increased 11-fold when the treatment was carried out in the presence of 2 mM GSH suggests that GSH may be able to slow the entry of Cd²⁺ into the cells by its ability to form a complex and thereby restrict transport capacity.

To determine whether Cd²⁺ was a topoisomerase II α poison we investigated cadmium chloride-induced DNA double strand breaks and cross resistance to K/VP.5 cells, a K562-derived cell line with decreased levels of topoisomerase II α mRNA and protein that is 26-fold resistant to the topoisomerase II poison etoposide [29]. While the K/VP.5 cells were only slightly cross-resistant to cadmium chloride (Fig. 3), cadmium chloride treatment of purified topoisomerase II α did not induce formation of DNA double strand breaks (Fig. 2). Thus, we conclude that poisoning of topoisomerase II by cadmium chloride does not contribute to inhibition of K562 cell growth. It should also be noted that it is possible that the effects of Cd²⁺ on K/VP.5 compared to K562 cells were masked due to other non-topoisomerase II-mediated mechanisms of cell growth inhibition. While some other thiol-reactive agents such as 1,4-benzoquinone [14] and etoposide orthoquinone [15] are reported to act as topoisomerase II α poisons, we found no evidence for such activity with either thimerosal [11] or cisplatin [10], two other heavy metal-containing compounds, though they also both strongly inhibited the catalytic activity of topoisomerase II α .

Both topoisomerase II poisons and catalytic inhibitors are well known to be cytotoxic by virtue of their interference with the critical role that topoisomerase II plays in cell division

[18-22]. It is also well known that anticancer topoisomerase II poisons (e.g. etoposide, teniposide, doxorubicin, mitoxantrone and others) can induce secondary malignancies by virtue of their ability to target topoisomerase II [20,23]. It is likewise well known that the clinically used catalytic topoisomerase II inhibitors such as dexrazoxane, razoxane and sobuzoxane are also carcinogenic and leukemogenic [23,24]. Thus, the fact that Cd^{2+} only acted as a catalytic inhibitor does not rule out that it may be carcinogenic through its ability to inhibit topoisomerase II. In conclusion, this study has shown that cadmium chloride is a potent inhibitor of topoisomerase II α activity and that this inhibition can be partially prevented by GSH which suggests that Cd^{2+} inhibits topoisomerase II by binding to critical cysteine residues. Even though cadmium chloride is not a topoisomerase II poison, catalytic inhibition of topoisomerase II may contribute, in part, to its cytotoxic, genotoxic and carcinogenic effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Canadian Institutes of Health Research, the Canada Research Chairs Program and a Canada Research Chair in Drug Development for B.B.H.; and by a grant to J.C.Y. from the NIH (grant CA090787).

Role of the funding source

The study sponsors had no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Abbreviations

α-MEM	alpha minimum essential medium
BSO	buthionine sulfoximine
GSH	glutathione kDNA kinetoplast DNA
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium
OTC	(-)-2-oxo-4-thiazolidinecarboxylic acid.

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Fig. 1.

(A) Inhibition of the decatenation activity of topoisomerase II α by cadmium chloride and the affect of GSH on the inhibition as measured in a fluorescence assay. The fluorescence measures the amount of decatenated kDNA minicircles in the supernatant of the centrifuged assay mixture. The reaction mixture contained no added GSH ($\circ$) or 500 μ M GSH ($\bullet$). The smooth lines are from non-linear least squares fits to a 4-parameter logistic equation. In the absence and presence of GSH the curve fitting yielded IC_{50} values of 26 ± 4 μ M and 149 ± 69 μ M, respectively. A limit of the number of samples that can be handled in a single experiment do not allow for multiple replicates for each cadmium chloride treatment. Thus, while the result shown is from a single experiment, results from 3 replicate determinations on different days gave an average 4.6 ± 0.6 -fold increase in the IC_{50} value in the presence of added GSH. (B) Fluorescent image of a gel showing inhibition of the decatenation activity of topoisomerase II α by cadmium chloride and the effect of GSH on this inhibition. Curve fitting yielded an IC_{50} of 50 μ M and 122 μ M in the absence and presence of GSH, respectively. In this ethidium bromide gel assay topoisomerase II α was present in the reaction mixture for all lanes but lanes 1 and 10. In the absence of topoisomerase II α the extremely high molecular weight catenated kDNA did not move from the origin. When the decatenation was highly inhibited, as in lanes 8, 9 and 18, most of the kDNA remained at the origin. In the other lanes, where there is only partial or no inhibition of topoisomerase II, there are higher order catenanes (containing 2, 3, 4 or more catenated minicircles) that are present that move slower on the gel than the fully decatenated kDNA. ORI is the gel origin,

NC is nicked circular DNA and OC is open circular DNA. In the absence of topoisomerase II α in the assay mixture the highly catenated kDNA does not move from the origin.

Fig. 2.

Fluorescent image of an ethidium bromide-stained gel showing the effect of cadmium chloride and etoposide on the topoisomerase II α -mediated cleavage of supercoiled (SC) pBR322 plasmid DNA. Topoisomerase II α (Topo II α) was present in the reaction mixture for all lanes but lane 2. As shown in lane 1, etoposide treatment produced significant amounts of linear DNA (LIN). A small amount of nicked circular (NC) is normally present in the pBR322 DNA. A densitometric analysis of the linear DNA bands showed that treatment with either 0.1 or 1 mM cadmium chloride produced much less linear DNA than the etoposide positive control (lane 1) and amounts of linear DNA about equal to that of topoisomerase II α alone (lane 3). At the high concentration of topoisomerase II used in this assay topoisomerase II induced catenation of the pBR322 DNA to form catenated DNA which can now be seen at the gel origin (ORI). In this ethidium bromide gel relaxed DNA (RLX) ran slightly ahead of supercoiled DNA.

Fig. 3.

Comparison of the effects of cadmium chloride on the growth inhibitory effects on a K562 and K/VP.5 cells with reduced levels of topoisomerase II. K562 ($\circ$) and K/VP.5 ($\bullet$) cells were treated with cadmium chloride for 72 h prior to the assessment of growth inhibition by an MTS assay. The curved lines are non-linear least squares fits to logistic equations and yield IC_{50} values of 19.0 ± 0.9 and $12.2 \pm 0.4 \mu M$, respectively for K562 and K/VP.5 cells.

Fig. 4.

Effect of reducing intracellular GSH levels in K562 cells increases the growth inhibitory effects of cadmium chloride. K562 cells were preincubated either without ($\circ$), or with 5 mM OTC ($\square$), or with 100 μ M BSO ($\bullet$) for 24 h and then treated for 72 h with cadmium chloride. The data shown are an average of 3 replicates. One other experiment performed on a different day with a BSO treatment yielded similar results. The slightly lower absorbance for both OTC and BSO treatments indicates that these compounds slightly reduced the growth of the K562 cells. The curved lines are non-linear least squares fits to 4-parameter logistic equations and yield IC_{50} values of 18 ± 4 , 0.77 ± 0.12 , and 23 ± 4 μ M for K562 cells not treated with BSO, or treated with BSO, or treated with OTC, respectively. Error bars are SEs.