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Doxorubicin inhibits human DNA topoisomerase I*

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Summary. Purified human DNA topoisomerase I was assayed quantitatively by enzyme titrations with supercoiled pHC624 DNA in the presence of 0–2.0 μM doxorubicin. Supercoiled and relaxed DNAs were resolved by agarose gel electrophoresis in the presence of ethidium bromide, and the percentage of conversion of supercoiled DNA to relaxed DNA was quantified by scanning microdensitometry. The inhibition of DNA topoisomerase I activity was measured at varying concentrations of doxorubicin. Doxorubicin inhibited enzyme activity at an IC_{50} value (the concentration required to inhibit 50% of the total activity) of 0.8 μM. Similar inhibition was observed for daunomycin, a structurally related anthracycline antitumor drug. These results indicate that anthracyclines inhibit human DNA topoisomerase I activity at concentrations that cause DNA damage and cytotoxicity in vivo.

Introduction

Type I DNA topoisomerases (Topo I) insert transient breaks into one strand of duplex DNA, whereas type II DNA topoisomerases (Topo II) break and rejoin both strands of duplex DNA [19]. Type II enzymes hydrolyze adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and inorganic phosphate (P_i) during enzyme turnover. Several effectors have been shown to inhibit both type I and type II DNA topoisomerases: ATP analogs, novobiocin, and coumermycin A_1 [2, 7, 13, 15]. These agents inhibit type II enzymes by binding to the ATP-binding site on the enzyme; the mechanism by which they inhibit type I topoisomerases is not known. The antibiotics oxolinic acid (Foglesong R/N, unpublished data) and nalidixic acid [15] inhibit vaccinia virus DNA Topo I. These drugs inhibit *Escherichia coli* DNA Topo II by binding to its catalytic subunit [13]. Several classes of antitumor drugs have been shown to inhibit type II DNA topoisomerases: anthracyclines, ellipticines, and amsacrines [12]. These drugs are intercalating agents that inhibit type II DNA topoisomerases by stabilizing a cleavable complex between the enzyme and the DNA. Another antitumor drug, camptothecin inhibits type I DNA topoisomerases via an analogous mechanism [10]. The epipodophyllotoxin antitumor drugs also inhibit type II DNA topoisomerase activity [12]. These drugs may weakly intercalate into duplex DNA, thereby stabilizing DNA Topo II-cleavable complexes (Pommier, personal communication).

In the present study, the effects of the anthracycline antitumor drugs doxorubicin and daunomycin on the catalytic activity of human DNA Topo I were investigated. Topo I activity was quantified by enzyme titrations in the presence and absence of drugs as described elsewhere [7].

Materials and methods

Materials. Doxorubicin (Cetus) was kindly supplied by Dr. A. Evans (Children's Hospital of Philadelphia). Daunomycin and doxorubicin were obtained from Sigma Chemical Company. Plasmid pHC624 was kindly provided by Dr. M.-A. Bjornsti (Thomas Jefferson University) and was used to transform *E. coli* DH5α (Bethesda Research Laboratories) according to the manufacturer's instructions. Supercoiled DNA was purified from the transformants as previously described [4]. Human DNA Topo I was purified to homogeneity from the yeast clone YEpGAL1-hTOP1 [1] and was kindly provided by Dr. M.-A. Bjornsti (Thomas Jefferson University). Another isolate of human DNA Topo I, which was purified to near homogeneity from HeLa cells, was obtained from Natra Cure.

Assay of DNA Topo I activity. Topo I (0.1 mg protein/ml) purified from YEpGAL1-hTOP1 was diluted 1 : 10 in 70 mM potassium phosphate (pH 7.5), 0.5 mM dithiothreitol, 0.5 mM ethylenediaminetetraacetic acid (EDTA), and 50% glycerol and was then incubated in 25 μl reactions containing 20 mM TRIS-HCl (pH 7.5), 150 mM, KCl, 10 mM EDTA, 1 mM 2-mercaptoethanol, 10% glycerol, and 0.8 μg supercoiled pHC624 DNA at 30° C for 60 min. Topo I (16 ng protein/ml) purified from HeLa

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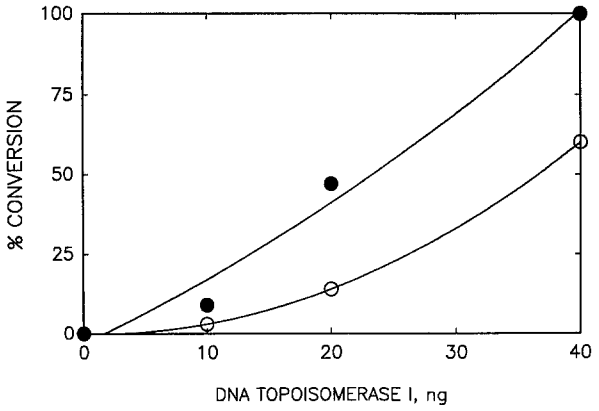

Fig. 1. Effect of doxorubicin the activity of human DNA topoisomerase I. Human DNA topoisomerase I was assayed in the presence (○) and absence (●) of 0.5 μM doxorubicin

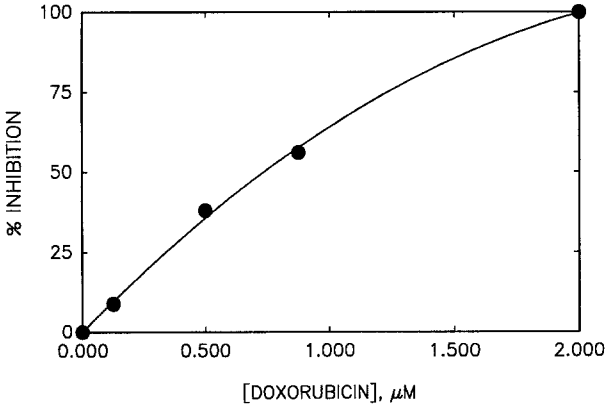

Fig. 2. Inhibition of human DNA topoisomerase I activity by doxorubicin. Human DNA topoisomerase I was assayed in the presence of 0–2.0 μM doxorubicin. The percentage of inhibition equals 100% minus the percentage of conversion of supercoiled DNA to relaxed DNA

cells was diluted 1 : 10 in 100 mM potassium phosphate (pH 7.0), 5 mM dithiothreitol, 1 mM EDTA, 1 mM sodium metabisulfite, 1 mM benzamide HCl, 0.2 mM phenylmethylsulfonyl fluoride, 1 μM leupeptin, 1 μM pepstatin A, and 50% glycerol and was then incubated in 25 μl reactions containing 50 mM TRIS-HCl (pH 7.5), 50 mM KCl, 10 mM $MgCl_2$, 1 mM EDTA, 5 mM dithiothreitol, 30 μl bovine serum albumin, and 0.8 μg supercoiled pHC624 DNA at 37° C for 30 min. All reactions were terminated by the addition of 0.17 mg proteinase K/ml, 0.17% sodium dodecyl sulfate, 21 mM EDTA, 0.002% bromophenol blue, and 1.7% glycerol followed by incubation at 37° C for 15 min. DNA was electrophoresed on 1% agarose submarine gels in 80 mM TRIS-acetate (pH 8.3), 2 mM EDTA, and 5 μg ethidium bromide/ml at 33 V for 4 h with circulation of the reservoir buffer. DNA bands were visualized under illumination from a shortwave UV light and were photographed with a Polaroid MP-3 camera using Polaroid type 55 film. The developed negatives were scanned with a Hoefer GS 300 scanning microdensitometer, and the areas under the peaks were determined using Hoefer Gelscan software. One unit of DNA topoisomerase activity is defined as the amount of enzyme activity that converts 50% of dimeric pHC624 supercoiled DNA to relaxed DNA under standard reaction conditions.

Results

Human DNA Topo I (10–40 ng) purified from yeast clone (YEpGAL1-hTOP1 was assayed as described in Materials and methods in the absence and presence of 0.5 μM doxorubicin (Adriamycin). The results are shown in Fig. 1. In the absence of drug, 22 ng Topo I exhibited 1.0 unit of activity. However, in the presence of 0.5 μM doxorubicin, 35 ng Topo I yielded 1.0 unit of activity. Therefore, 0.5 μM doxorubicin inhibited 39% of Topo I activity. Control experiments were performed in which pBR322 DNA was completely relaxed by incubation with human Topo I, after which doxorubicin was added to concentrations of 0.1–3.0 μM and electrophoresis was carried out in the presence of 0.5 μg ethidium bromide/ml as described in Materials and methods. No shift in DNA migration was observed (data not shown), indicating that doxorubicin concentrations below 3.0 μM do not affect the electrophoretic migration of DNA under the conditions used in this study to quantify Topo I activity.

Topo I assays were performed at several concentrations of doxorubicin (from 0.125 to 2.0 μM). The percentage of inhibition of the enzyme was calculated for each incubation dose as described above. The results are shown in Fig. 2. Inhibition of Topo I activity increases with increasing doxorubicin concentration. The IC_{50} value for doxorubicin as determined in this analysis was 0.8 μM. This value is much lower than the IC_{50} values reported for inhibitors of vaccinia virus Topo I, the eukaryotic DNA Topo I for which drug inhibition is best characterized: 42 μM for coumermycin A_1, 180 μM for novobiocin, 1.0 mM for β, γ-imido ATP [7], and 460 μM for oxolinic acid (Foglesong, unpublished data). Berenil inhibits the activity of vaccinia Topo I by 65% at a concentration of 4 μM [15] and also inhibits rat-liver mitochondrial (but not nuclear) Topo I [6]. Complete inhibition of human Topo I activity was observed in the presence of 2.0 μM doxorubicin. Similar results were obtained for daunomycin (data not shown). Doxorubicin at this concentration has been shown to induce an equal ratio of single- and double-strand DNA breaks with protein covalently attaching to the ends of the DNA and to cause a 100-fold reduction in the survival of murine L1210 cells [21]. These results are consistent with the hypothesis that the cytotoxicity upon treatment to cells with anthracyclines is caused by DNA damage due to the stabilization of DNA topoisomerase-cleavable complexes [12].

DNA Topo I purified from HeLa cell nuclei was also assayed in the presence of doxorubicin as described above. There was no detectable difference in the inhibition of this isolate of human Topo I with respect to doxorubicin inhibition (data not shown). This result indicates that if there are differences in Topo I posttranslational modification between human and yeast cells, they do not affect its sensitivity to doxorubicin.

The results of this study indicate that the anthracycline antitumor drugs doxorubicin and daunomycin are potent inhibitors of human type I DNA topoisomerase. It has been reported, albeit without substantiating data, that doxorubicin inhibits calf-thymus Topo I [18]. In addition the inhibition of murine L1210 cell Topo I by micromolar concentrations of doxorubicin and 3′-deamino-3′ (4-morpholinyl)-doxorubicin has been reported [20]. We have recently described the inhibition of vaccinia virus DNA Topo I by doxorubicin and daunomycin (Foglesong et al., submitted for publication). Therefore, the anthracycline antitumor drugs have been shown to inhibit several type I DNA

topoisomerases of both cellular and viral origin. These results suggest that inhibition of Topo I may be a primary mechanism of anthracycline cytotoxicity since Topo I is present in the nucleus at levels at least 10-fold those of Topo II [18].

Discussion

The mechanism underlying the inhibition of DNA topoisomerase activity by anthracyclines is not clear. At a concentration of 1 μM doxorubicin only slightly altered the sequence-specific DNA cleavage catalyzed by murine Topo I [20]. Altered DNA cleavage would support the hypothesis that the mechanism by which anthracyclines inhibit Topo I activity involves DNA intercalation within preferred cleavage sites of the enzyme and stabilization of a cleavable complex, which is analogous to the pathway of inhibition of type II DNA topoisomerase activity by doxorubicin and other intercalating agents [12]. However, in this study no increase in nicked DNA was observed for human Topo I in the presence of inhibiting concentrations of anthracyclines, as would be expected from the stabilization of cleavable complexes (data not shown). Altered DNA cleavage by calf-thymus Topo II has clearly been demonstated in the presence of micromolar concentrations of doxorubicin and daunomycin [17]. The cleavage of DNA by murine Topo I is generally inhibited at a concentration of 10 μM doxorubicin or its morpholinyl derivatives [18]. This finding was interpreted as a reflection of decreased binding of the enzyme to DNA in the presence of relatively high concentrations of intercalating agents. Alternatively, anthracyclines may bind to an allosteric site on Topo I, thereby inhibiting its activity. This mechanism of Topo I inhibition has been proposed for ATP analogs, novobiocin, and coumermycin A_1 [2, 7], which are known to bind to the ATP-binding site of Topo II [13]. Further studies are in progress to elucidate the molecular mechanism underlying doxorubicin's inhibition of human Topo I activity.

Several doxorubicin-resistant mammalian cell lines have been isolated, some of which express an altered 185-kDa P-glycoprotein, which functions in drug efflux [9]. Some, however, show altered DNA topoisomerase II [3, 5, 11, 14]. This supports the hypothesis that Topo II is the target of doxorubicin in vivo. No Topo I mutant has been described among the doxorubicin-resistant cell lines characterized. However, several Topo I mutants have been isolated as cells that are resistant to the antitumor drug camptothecin [8, 16] which inhibits purified Topo I in vitro [10]. The cross-resistance of these cells to other antitumor drugs has been characterized for five of these mutants; four of the cell lines showed increased sensitivity to doxorubicin, but the A549/CPT line exhibited 2.3-fold cross-resistance to doxorubicin [16]. This human lung-cancer cell line contains normal levels of Topo I, unlike the other camptothecin-resistant cell lines, which exhibit reduced levels of Topo I. The cross-resistance of A549/CPT to doxorubicin is consistent with the hypothesis that Topo I is a physiological target for doxorubicin in this cell line. Therefore, the inhibition of human Topo I by doxorubicin may be relevant to the cytotoxicity of this drug in some human cells.

References

1. Bjornsti M-A, Benedetti P, Viglianti GA, Wang JC (1989) Expression of human DNA topoisomerase I in yeast cells lacking yeast DNA topoisomerase I: restoration of sensitivity of the cells to the antitumor drug camptothecin. Cancer Res 49: 6318
2. Castora FJ, Kelly WG (1986) ATP inhibits nuclear and mitochondrial type I topoisomerases from human leukemia cells. Proc Natl Acad Sci USA 83: 1680
3. Cole SPC, Chanda ER, Dicke FP, Gerlach JH, Mirski SE (1991) Non-P-glycoprotein-mediated multidrug resistance in a small-cell lung cancer cell line: evidence for decreased susceptibility to drug-induced DNA damage and reduced levels of topoisomerase II. Cancer Res 51: 3345
4. Davis LG, Dibner MD, Battey JF (1986) Plasmid DNA preparation: triton-lysozyme method: In: Basic methods in molecular biology. Elsevier, Amsterdam, p 93
5. Deffie AM, Batra JK, Goldenberg GJ (1989) Direct correlation between DNA topoisomerase II activity and cytotoxicity in Adriamycin-sensitive and -resistant P388 leukemia cell lines. Cancer Res 49: 58
6. Fairfield FR, Bauer WR, Simpson MV (1979) Mitochondria contain a distinct DNA topoisomerase. J Biol Chem 254: 9352
7. Foglesong PD, Bauer WR (1984) Effects of ATP and inhibitory factors on the activity of vaccinia virus type I topoisomerase. J Virol 49: 1
8. Gupta RS, Gupta R, Eng B, Lock RB, Ross WE, Hertzberg RP, Caranfa MJ, Johnson RK (1988) Camptothecin-resistant mutants of Chinese hamster ovary cells containing a resistant form of topoisomerase I. Cancer Res 48: 6404
9. Horio M, Gottesman MM, Pastan I (1988) ATP-dependent transport of vinblastine in vesicles from human multidrug-resistant cells. Proc Natl Acad Sci USA 85: 3580
10. Hsiang Y-H, Hertzberg R, Hecht S, Liu LF (1985) Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. J Biol Chem 260: 14873
11. Jong S de, Zijlstra JG, Vries EGE de, Mulder NH (1990) Reduced DNA topoisomerase II activity and drug-induced DNA cleavage activity in an Adriamycin-resistant human small-cell lung carcinoma cell line. Cancer Res 50: 304
12. Liu LF (1989) DNA topoisomerases poisons as antitumor drugs. Annu Rev Biochem 58: 351
13. Maxwell A, Gellert M (1986) Mechanistic aspects of DNA topoisomerases. In: Anfinsen CB, Epsall JT, Richards FM (eds) Advances in protein chemistry, vol 38. Academic Press, San Diego, p 69
14. Pommier Y, Kerrigan D, Schwartz RE, Swack JA, McCurdy A (1986) Altered DNA topoisomerase II activity in Chinese hamster cells resistant to topoisomerase II inhibitors. Cancer Res 46: 3075
15. Shaffer R, Traktman P (1987) Vaccinia virus encapsulates a novel topoisomerase with the properties of a eucaryotic type I enzyme. J Biol Chem 262: 9309
16. Sugimoto Y, Tsukahara S, Oh-Hara T, Isoe T, Tsuruo T (1990) Decreased expression of DNA topoisomerase I in camptothecin-resistant tumor cell lines as determined by a monoclonal antibody. Cancer Res 50: 6925
17. Tewey KM, Rowe TC, Yang L. Halligan BD, Liu LF (1984) Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. Science 226: 466
18. Vosberg H-P (1986) DNA topoisomerases: enzymes that control DNA conformation: In: Cooper M, Eisen H, Goebel W, Hofschneider PH, Koprowski H, Melchers F, Oldstone M, Rott R, Schweiger HG, Vogt PK, Wilson I (eds) Current topics in microbiology and immunology, vol 114. Springer, Berlin Heidelberg New York, p 19
19. Wang JC (1985) DNA topoisomerases. Annu Rev Biochem 54: 665
20. Wasserman K, Markovits J, Jaxel C, Capranico G, Kohn K, Pommier Y (1990) Effects of morpholinyl doxorubicins, doxorubicin, and actinomycin D on mammalian DNA topoisomerases I and II. Mol Pharmacol 38: 38
21. Zwelling LA, Michaels S, Erikson LC, Ungerleider RS, Nichols M, Kohn KW (1981) Protein-associated deoxyribonucleic acid strand breaks in L1210 cells treated with the deoxyribonucleic acid intercalating agents 4′-(9-acridinylamino) methanesulfon-*m*-anisidide and Adriamycin. Biochemistry 20: 6553