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Suramin is an inhibitor of DNA topoisomerase II *in vitro* and in Chinese hamster fibrosarcoma cells

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ABSTRACT **The antitrypanosomal and antifiliarial drug suramin is currently under investigation for treatment of advanced malignancies including prostatic cancer, adrenocortical cancer, and some lymphomas and sarcomas. Here we show that suramin is a potent inhibitor of the nuclear enzyme DNA topoisomerase II. Suramin inhibited purified yeast topoisomerase II with an IC_{50} of about 5 μM, as measured by decatenation or relaxation assays. Suramin did not stabilize the covalent DNA–topoisomerase II reaction intermediate ("cleavable complex"), whereas other inhibitors of this enzyme, such as amsacrine, etoposide, and the ellipticines, are known to stabilize the intermediate. In contrast, the presence of suramin strongly inhibited the cleavable-complex formation induced by amsacrine or etoposide. Accumulation of the endogenous cleavable complex was also inhibited. Suramin entered the nucleus of DC-3F Chinese hamster fibrosarcoma cells exposed to radiolabeled suramin for 24 hr as shown by both optic and electron microscopy. The suramin present in the nucleus seemed to interact with topoisomerase II, since suramin reduced the number of amsacrine-induced protein-associated DNA strand breaks in DC-3F cells and protected these cells from the cytotoxic action of amsacrine. Cells resistant to 9-hydroxyellipticine, which have been shown to have an altered topoisomerase II activity, are about 7-fold more resistant to suramin than the sensitive parental cells as shown by 72-hr growth inhibition assay. Our results suggest that DNA topoisomerase II is a target of suramin action and that this action may play a role in the cytotoxic activity of suramin.**

Suramin is a hexasulfated naphthylurea that has been used in the treatment of trypanosomiasis (sleeping sickness) and onchocerciasis for more than half a century (1). More recently, suramin was shown to prevent infection of T lymphocytes by human immunodeficiency virus *in vitro* (2). This led to clinical trials of the compound in AIDS patients, where it showed little therapeutic activity (3, 4). However, during these trials a complete clinical response was noted in a patient with Kaposi sarcoma and non-Hodgkin lymphoma (4), and subsequent studies showed that suramin was active in the treatment of several metastatic cancers such as renal cancer, adrenocarcinoma, lymphoma, and prostate cancer (5, 6).

Suramin inhibits the binding of platelet-derived growth factor, epidermal growth factor, basic fibroblast growth factor, transforming growth factor β, and insulin-like growth factor to their specific cell surface receptors (7–11). The drug also affects the activity of protein kinase C and inhibits phosphatidylinositol kinase and diacylglycerol kinase (12, 13). Several nuclear enzymes are also inhibited by suramin. These include DNA and RNA polymerases, terminal deoxynucleotidyltransferase, and reverse transcriptase (14–17). Suramin can induce cell differentiation in several different systems (18, 19) and can inhibit tumor cell invasion (20).

Here we demonstrate that suramin is a potent inhibitor of the nuclear enzyme DNA topoisomerase II, which is a known target for a number of currently used antineoplastic agents. We show that suramin interacts with the enzyme in living cells and that this interaction may play a role in the cytotoxic activity of suramin.

MATERIALS AND METHODS

Enzymes. DNA topoisomerase II was obtained from *Saccharomyces cerevisiae* as described (21). Briefly, DNA topoisomerase II was overexpressed in yeast from a multicopy expression plasmid kindly provided by James Wang (Harvard University). The purified enzyme preparation contained no detectable DNA topoisomerase I activity.

DNA Substrates. Supercoiled plasmid pBR322 DNA (>95% form I) and calf thymus DNA were purchased from Boehringer Mannheim. Highly catenated kinetoplast DNA (form I) was purified from *Trypanosoma cruzi* (provided by Guy Riou, Villejuif, France) after DNA extraction and sucrose sedimentation (22).

Antitumor Drugs. Amsacrine [4′-(9-acridinylamino)-3-methanesulfon-*m*-anisidide] was a gift from Bruce Baguley (Auckland Medical School, Auckland, New Zealand). [^{14}C]Amsacrine (>99% pure; 20.8 mCi/mmol; 1 mCi = 37 MBq) was provided by the Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, MD. Suramin (Bayer) and etoposide (VP-16-213) were gifts from J.-P. Armand (Villejuif, France) and W. T. Bradner (Bristol–Myers Squibb, Syracuse, NY), respectively. [^{3}H]Suramin (9 Ci/mmol) was purchased from Moravek Biochemicals (Brea, CA). The radiochemical purity was >98% as determined by HPLC (23).

Relaxation Assay. The reaction mixture contained 50 mM Tris·HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 165 mM KCl, 1 mM ATP, and 150 ng of pBR322 DNA. The reaction was initiated by the addition of DNA topoisomerase II and allowed to proceed at 30°C for 10 min. Reactions were terminated by addition of SDS, bromophenol blue, and sucrose (1%, 0.05%, and 10% final concentrations, respectively). The samples were electrophoresed in 1% agarose gels at 2 V/cm for 18 hr in Tris/borate/EDTA buffer at pH 8. Photographic negatives of the ethidium bromide-stained agarose gels were scanned with a Joyce–Loebl Chromoscan 3 densitometer and the peak areas of supercoiled DNA were determined.

Decatenation Assay. Reaction conditions were as described above except that 200 ng of kinetoplast DNA was used as substrate instead of pBR322, and the incubation was for 15 min. Electrophoresis was in 1.2% agarose gels at 5 V/cm for

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4 hr. Liberated minicircles were quantified by densitometric scanning of photographic negatives.

Formation of Cleavable Complex. The experimental conditions were the same as for the relaxation except that about 50-fold more DNA topoisomerase II was used. For the assays containing both suramin and amsacrine or etoposide, pBR322 DNA was added first, followed by amsacrine/etoposide and then suramin; the reaction was initiated with the addition of enzyme. After 20 min at 30°C the reactions were terminated by addition of 1% SDS and 0.1% proteinase K followed by incubation at 50°C for 30 min. Electrophoresis in 1% agarose gels containing ethidium bromide (0.5 μg/ml) was carried out at 2 V/cm for 18 hr in Tris/borate/EDTA buffer (pH 8) with ethidium bromide (0.5 μg/ml). All topoisomerase II assays were done at least twice with two different enzyme preparations.

DNA Interactions. Binding of suramin to DNA was tested in thermal denaturation experiments (24) on a computerized Kontron 940 spectrophotometer with a twelve-cell changer.

Cells and Culture Medium. The Chinese hamster cell line DC-3F and the 9-hydroxyellipticine-resistant subline DC-3F/9-OH-E have been described (25), as have the media and growth conditions (25, 26).

Localization of Suramin in Chinese Hamster Cells. The intracellular location of suramin was determined by autoradiography of whole cells and cellular sections. The cells were incubated for 24 hr in medium containing [^{3}H]suramin (50 μM, specific activity, 1 Ci/mmol), rinsed, and fixed with 1.6% glutaraldehyde in Sörensen's phosphate buffer (0.1 M phosphate, pH 7.3) at 4°C for 30 min. Control cells were incubated for 24 hr in growth medium, rinsed, and treated momentarily with [^{3}H]suramin-containing medium, which was immediately withdrawn. The cells were prepared for autoradiography as described (27, 28).

For quantitative analysis of autoradiographs, all cells in a section were photographed and the magnification was checked by comparison with a grating replica (Fullam, Schenectady, NY). The grains were counted, and the nuclear and cytoplasmic areas of the cells were determined with a graphic digitizer (Hewlett–Packard). Thirty-five cells were analyzed in this manner.

Measurement of DNA Damage by Alkaline Elution. This method has been described (29). Elution of DNA was carried out under DNA-denaturing (pH 12.1) and nondeproteinizing conditions. DNA–protein crosslinks were calculated by using the bound-to-one-terminus model (29).

Drug Accumulation. Drug uptake in DC-3F and DC-3F/9-OH-E cells was determined as described (30, 31).

Flow Cytometry. Flow cytofluorimetric analysis of the DNA content in isolated nuclei was carried out as described (28) with a model 2103 flow cytometer (Ortho Instruments) coupled to an IBM PC-AT computer. The relative fractions of cells in the different phases of the cycle were determined according to the model of Dean and Jett (32).

Cytotoxicity. Experiments were carried out with exponentially growing cells (26, 27).

RESULTS

Suramin Inhibits the Catalytic Activities of Purified Yeast DNA Topoisomerase II *in Vitro*. The effect of suramin on the catalytic activity of purified DNA topoisomerase II was assayed by two methods. Suramin inhibited the ability of topoisomerase II to relax supercoiled DNA with an IC_{50} of ≈4 μM (Fig. 1). Suramin also inhibited the decatenation of trypanosomatid kinetoplast DNA by topoisomerase II, with an IC_{50} of ≈7 μM (results not shown).

Suramin Inhibits Cleavable-Complex Formation. Topoisomerase II-mediated DNA strand passage requires breakage and rejoining of the double-stranded DNA. During this process, the enzyme becomes covalently linked to the 5′ phosphate of both DNA strands via a tyrosine-DNA phosphodiester linkage (33, 34). The covalent reaction intermediate is called the cleavable complex and can be demonstrated experimentally by the enzyme-dependent formation of linear DNA from supercoiled DNA after treatment with SDS and proteinase K (35). Clinically used DNA topoisomerase II inhibitors such as amsacrine, anthracyclines, epipodophyllotoxins, and ellipticine derivatives act by stabilizing the cleavable complex (36–39). Suramin at concentrations up to 75 μM did not induce DNA cleavage (Fig. 2, lanes 4–6). In contrast, 10 μM suramin completely inhibited the endogenous cleavable-complex formation (compare lane 4 with lane 3). We then examined whether suramin was also able to inhibit amsacrine- or etoposide-induced cleavable complex formation. As little as 10 μM suramin completely inhibited the DNA cleavage induced by 75 μM amsacrine (compare lanes 7 and 8). A similar effect of suramin was observed on etoposide-induced cleavable complexes (lanes 11–14). DNA cleavage induced by 75 μM etoposide was inhibited by suramin in a concentration-dependent manner (lanes 12–14).

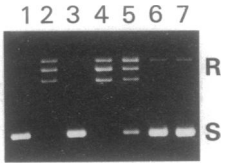

FIG. 1. Inhibition of the catalytic activity of purified yeast DNA topoisomerase II by suramin as measured by relaxation. Supercoiled pBR322 DNA (lane 1) was relaxed by purified topoisomerase II in the absence (lane 2) or presence of 2, 5, 10, or 20 μM suramin (lanes 4–7). Plasmid pBR322 was also incubated with 20 μM suramin in the absence of topoisomerase II (lane 3). S, supercoiled; R, relaxed.

Suramin Binding to DNA. The ability of a compound to alter the thermal denaturation profile of DNA is used as an indication of the binding of the compound to DNA (24). The average value of the midpoint thermal denaturation temperature for calf thymus DNA was 61.2°C. The presence of ethidium bromide increased the thermal denaturation temperature to 76.3°C. In contrast, no significant change of the denaturation temperature was observed for suramin under the same experimental conditions.

Cellular Distribution of Suramin. Since DNA topoisomerase II is a nuclear enzyme, we wanted to determine whether suramin entered the nucleus. Fig. 3 *A* and *B* illustrate the intracellular distribution of [^{3}H]suramin in DC-3F Chinese hamster fibrosarcoma cells after 24 hr of incubation with 50 μM [^{3}H]suramin. Suramin at this concentration has no detectable effects on cell growth. Suramin appeared to be predominantly located in the nucleus as shown by whole cell autoradiography (Fig. 3*A*) and confirmed by autoradiographs of cellular sections (Fig. 3*B*). Control cells, which were exposed to [^{3}H]suramin only momentarily, showed no cell-

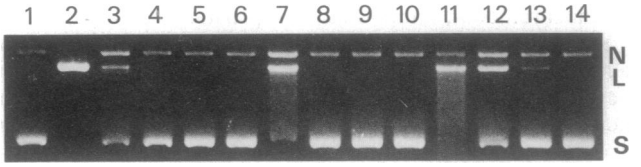

FIG. 2. Effect of suramin on DNA cleavage mediated by purified yeast DNA topoisomerase II. Purified topoisomerase II was incubated with supercoiled pBR322 DNA in the presence of suramin, amsacrine, or etoposide. Lane 1: supercoiled pBR322 DNA; lane 2: linear pBR322 DNA; lanes 3–6: 0, 10, 25, and 75 μM suramin; lanes 7–10: 75 μM amsacrine with 0, 10, 25, and 75 μM suramin; lanes 11–14: 75 μM etoposide with 0, 10, 25, and 75 μM suramin. S, supercoiled; L, linear; N, nicked.

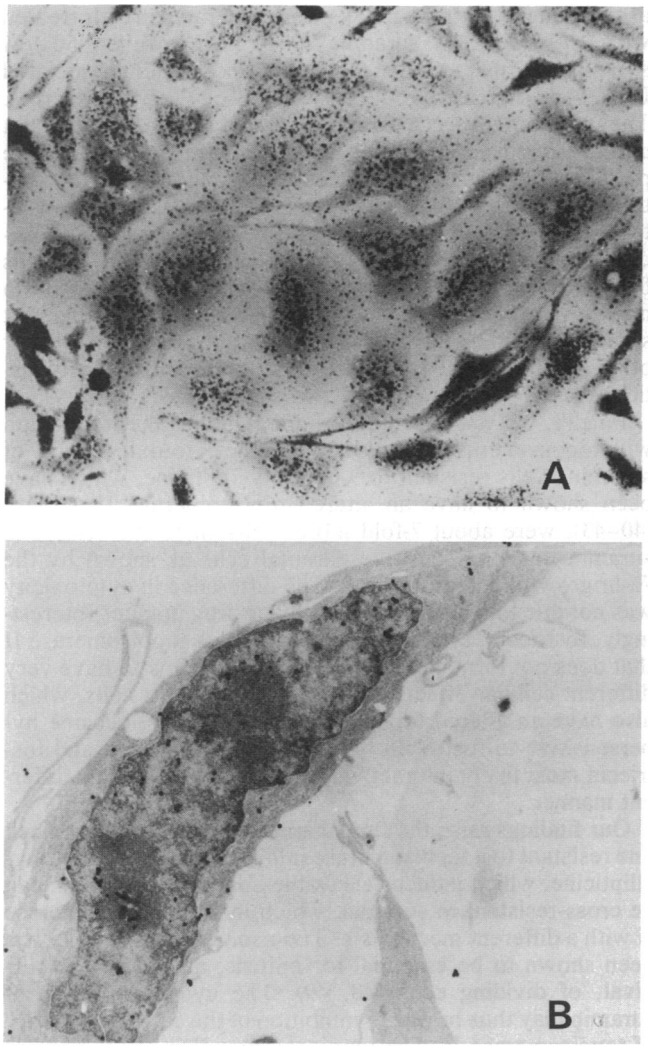

FIG. 3. Autoradiographs of Chinese hamster DC-3F cells exposed to [^{3}H]suramin (50 μM) for 24 hr. (*A*) Whole cells. (×365.) (*B*) Cellular sections. (×11,000.) Silver grains are predominantly located in the nuclear areas.

associated radioactivity above background (results not shown).

Quantitative determinations of the cellular distribution of suramin (based on 35 individual cells) showed that the density of silver grains was about 3 times greater over the nuclear area than over the cytoplasmic areas of the cell ($P < 0.001$, Student's t test): 2.87 ± 0.94 vs. 1.19 ± 0.66 (mean ± SD, arbitrary units).

Suramin Inhibits the Production of Protein-Linked DNA Strand Breaks Induced by Amsacrine in DC-3F Cells. The alkaline elution technique was used to determine the occurrence of protein-linked DNA strand breaks in DC-3F cells treated for 24 hr with suramin at doses up to 500 μM. We found that no protein-associated DNA strand breaks were induced even at the highest dose (results not shown). Since suramin strongly inhibited the cleavable complex formation induced by amsacrine *in vitro*, the occurrence of protein-linked DNA strand breaks induced by amsacrine in the absence and presence of suramin was also studied (Table 1). Suramin (50 μM) inhibited the amsacrine-induced formation of protein-linked DNA strand breaks. The decrease in protein-linked DNA strand breaks was not due to a decrease of amsacrine uptake in suramin-treated cells. Also, at this dose suramin has no detectable effects on thymidine incorporation and cell growth. In addition, no cell cycle pertubations were observed as determined by flow cytometry analysis of the DNA content in control and suramin-treated cells (results not shown).

Table 1. Frequencies of DNA–protein crosslinks produced by amsacrine in DC-3F Chinese hamster fibrosarcoma cells

	Crosslinks, rad eq.	
	100 nM amsacrine	200 nM amsacrine
Control	751	1127
50 μM suramin	309	367

Cells were grown in the absence or presence of suramin (50 μM) for 21 hr and then treated with amsacrine (100 or 200 nM) for 3 hr. Values (expressed in rad equivalents; 1 rad = 0.01 Gy) are the means of at least two independent experiments, each performed in duplicate.

Suramin Protects DC-3F Cells from the Cytotoxic Action of Amsacrine. The decrease of amsacrine-induced protein-linked DNA strand breaks in suramin-treated cells (Table 1) was accompanied by a decrease in the cytotoxic effect of amsacrine (Fig. 4). The ED_{50} of amsacrine for control DC-3F cells was 30 nM, whereas the ED_{50} for suramin-treated DC-3F cells was 70 nM.

Cytotoxicity of Suramin. The effect of suramin on the growth of the Chinese hamster cell line DC-3F and the 9-hydroxyellipticine-resistant subline DC-3F/9-OH-E is shown in Fig. 5. DC-3F/9-OH-E cells are cross-resistant to DNA topoisomerase II inhibitors such as etoposide and amsacrine and have been shown to have an altered DNA topoisomerase II activity (40–43). DC-3F/9-OH-E cells were about 7-fold more resistant to suramin than the parental DC-3F cells (Fig. 5); the ED_{50} for DC-3F was 31 μM whereas the ED_{50} for DC-3F/9-OH-E was 203 μM. The resistance of DC-3F/9-OH-E to suramin was not due to a reduced drug uptake, since the cellular uptake of suramin was the same for DC-3F and DC-3F/9-OH-E (results not shown).

DISCUSSION

A variety of biological systems have been shown to be affected by suramin (7–20). However, it is difficult to evaluate which, if any, of these targets is involved in the antitumor activity. Several of the effects have been demonstrated clearly for *in vitro* systems only, or at high suramin concen-

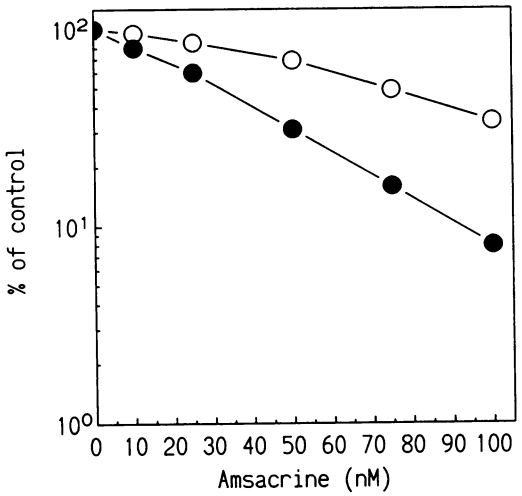

FIG. 4. Effect of suramin on amsacrine cytotoxicity in DC-3F cells. Control (●) or suramin-treated (○) DC-3F cells were grown in the absence or presence of suramin (50 μM) for 21 hr and then exposed to amsacrine for 3 hr. Cell survival was determined by colony formation. Each point represents an average of two independent experiments performed in duplicate.

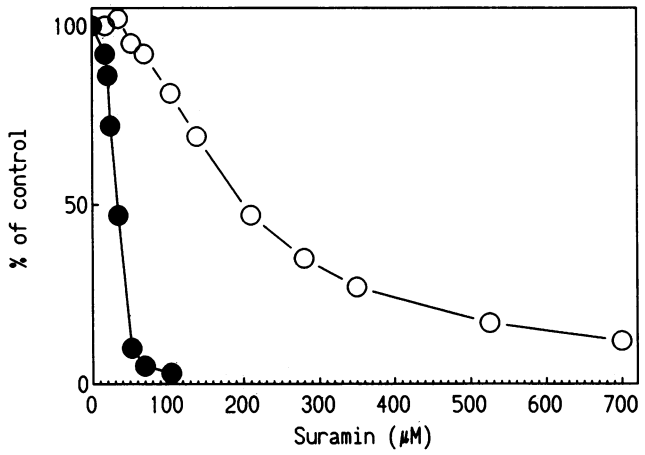

FIG. 5. Effect of suramin on the growth of DC-3F (○) and DC-3F/9-OH-E (●) cells. Cells were exposed to various concentrations of suramin for 72 hr. After drug removal, cells were trypsinized and counted. Each point represents an average of at least two independent experiments performed in duplicate.

trations. A further complication has been the lack of knowledge about the intracellular distribution of suramin.

Here we show that suramin is a potent inhibitor of the nuclear enzyme DNA topoisomerase II *in vitro*. Suramin penetrates to the nucleus of Chinese hamster fibrosarcoma cells, where it interacts with topoisomerase II. Our results further indicate that this interaction plays a role in the cytotoxic activity of suramin.

Suramin is a potent inhibitor of purified topoisomerase II with an IC_{50} of about 5 μM as measured by either decatenation or relaxation assays. Unlike amsacrine and etoposide, suramin did not induce any detectable formation of cleavable complex with purified topoisomerase II. In contrast, suramin strongly inhibited the amsacrine- or etoposide-induced cleavable-complex formation in a concentration-dependent manner. This appears to be a direct effect rather than due to drug–drug interactions between suramin and amsacrine or etoposide, since the endogenous formation of cleavable complex was inhibited as well.

Preliminary results in our laboratory suggest that suramin binds to topoisomerase II. This is probably due to electrostatic interactions between the enzyme, which is a basic protein, and the hexasulfated suramin. No suramin binding to DNA was detected, although low-affinity binding cannot be excluded. Further experiments are needed to establish whether the suramin–topoisomerase complex may react with DNA to form a noncovalent ternary complex or whether the topoisomerase II, once it is bound to suramin, is unable to react with DNA.

Because topoisomerase II is a nuclear enzyme, we wanted to determine whether suramin could enter the nucleus of living cells. Chinese hamster fibrosarcoma cells were exposed to radiolabeled suramin for 24 hr, and the intracellular localization of the drug was determined by autoradiography. The grain density was almost 3 times higher in the nucleus than in the cytoplasm. The radioactivity was most likely associated with intact suramin, since suramin is known not to be metabolized (44). Suramin is taken up by endocytosis and concentrated in the lysosomes (45). Interestingly, heparan sulfate is internalized in a similar manner, whereafter a part of the material is transported to the nucleus (46). Since suramin seems to act as an analog of heparan and dermatan sulfate with regard to a variety of enzyme systems (5), it is possible that suramin enters the nucleus by the same route as heparan sulfate.

We then wanted to know whether suramin present in the nucleus interacted with topoisomerase II. No protein-associated DNA strand breaks were generated in cells treated with up to 500 μM suramin for 24 hr. However, 50 μM suramin was able to reduce the number of protein-associated DNA strand breaks produced in DC-3F cells by amsacrine. The reduction of DNA strand breakage by suramin was not due to decreased amsacrine uptake or a result of cell cycle perturbations. Therefore, the effects of suramin on the protein-associated DNA strand breaks in living cells are consistent with the results obtained with purified enzyme. Subsequent studies showed that suramin protected the DC-3F cells from the cytotoxic effects of amsacrine. This observation may have important clinical implications by suggesting that suramin should not be used in combination with amsacrine or other topoisomerase II inhibitors in the treatment of neoplastic disease.

Finally, we wanted to determine whether the interaction with topoisomerase II plays a role in the cytotoxic activity of suramin. Cells resistant to 9-hydroxyellipticine, which have been shown to have an altered topoisomerase II activity (40–43), were about 7-fold more resistant to the effects of suramin than the sensitive parental cells as shown by the 72-hr growth inhibition assay. The difference in cytotoxicity was not due to a difference in cellular drug uptake. Interestingly, fostriecin, another inhibitor of DNA topoisomerase II that does not form cleavable complexes seems to have very different cellular effects. Doxorubicin-resistant cells, which also have an altered topoisomerase II activity, became hypersensitive to fostriecin (47). Therefore, suramin and fostriecin most likely interact with topoisomerase II in a different manner.

Our findings raise the fundamental question of how a cell line resistant to a topoisomerase inhibitor such as 9-hydroxyellipticine, which induces cleavable-complex formation, can be cross-resistant to suramin, which inhibits topoisomerase II with a different mechanism. Topoisomerase II activity has been shown to be essential for mitosis, and therefore survival, of dividing cells (48, 49). The cytotoxic effect of suramin may thus be due to inhibition of the catalytic activity of topoisomerase II. The topoisomerase II in the 9-hydroxyellipticine-resistant cells is most likely a mutant enzyme (42). This could result in a lower affinity for suramin, which would then make the cells more resistant to the drug.

Another possibility is that suramin and 9-hydroxyellipticine share some common elements in their cytotoxic action. Topoisomerase II exists in two forms, p170 and p180 (50). The p170 form is more sensitive to teniposide, which induces cleavable-complex formation, and to merbarone, which like suramin does not induce cleavable-complex formation (50). If the p170 form is also more sensitive to 9-hydroxyellipticine and suramin, 9-hydroxyellipticine-resistant cells are likely to contain less of this form and therefore become cross-resistant to suramin.

It is possible that binding of suramin to topoisomerase II might affect its association with the nuclear matrix. Topoisomerase II exists in a soluble (salt-extractable) and a matrix-associated form. The latter may play a role in the attachment of DNA loops to the nuclear matrix (51). It has been suggested that it is the nuclear matrix-associated topoisomerase II that is the cytotoxic target for amsacrine and other cleavable-complex inducers (52). If the association of topoisomerase II to the nuclear matrix is altered in the 9-hydroxyellipticine-resistant cell line, as is the case for human leukemia cells resistant to teniposide (53), this might result in a decreased sensitivity to suramin.

Our results show that suramin interacts with topoisomerase II in living cells and that this interaction plays a role in the cytotoxic activity of suramin. This finding may have several practical implications. Better knowledge of the mechanism of action of suramin may provide a rationale for the selection of agents that could be used, or that should not be used, in

association with suramin in the treatment of neoplastic disease. It may also be useful in the development of more-active suramin derivatives. Our results suggest that topoisomerase inhibitors other than cleavable-complex inducers may be clinically active antineoplastic agents.

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