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Chemoprotection by 9-Aminoacridine Derivatives against the Cytotoxicity of Topoisomerase II-directed Drugs*

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Abstract—*The effect of three acridine derivatives, 9-aminoacridine (9AA), 4'-(9-acridinylamino)-methanesulphon-o-anisidide (o-AMSA) and quinacrine were compared in their ability to protect against the cytotoxicity of amsacrine, 9-[[2-methoxy-4-[(methylsulfonyl)amino]phenyl]amino)-N,5-dimethyl-4-acridine-carboxamide (CI-921), N-[2-(dimethylamino)ethyl]acridine-4-carboxamide (AC), etoposide, mitoxantrone and doxorubicin. Cytotoxicity was measured* in vitro *by clonogenic survival assay and* in vivo *by life extension assays. All three acridine derivatives protected a Lewis lung cell line* in vitro *against CI-921, with 9AA having the highest activity. Cellular uptake of* [^{14}C]*CI-921 by cultured Lewis lung cells was unaffected by 9AA, and slightly stimulated by o-AMSA and quinacrine. 9AA protected Lewis lung cells* in vitro *against the cytotoxicity of amsacrine, CI-921, AC and etoposide, partially against mitoxantrone but not against doxorubicin. A similar result was obtained with the human melanoma cell line MM96, where 9AA protected against CI-921 but not against doxorubicin toxicity. 9AA protected P388 leukaemia* in vivo *against amsacrine, CI-921 and AC ctytotoxicity, partially against etoposide but not against mitoxantrone or doxorubicin. 9AA also protected against animal toxicity caused by high dose amsacrine and partially against CI-921 toxicity. It is hypothesized that DNA intercalating chemoprotectors act by restricting the conformational flexibility of the DNA and thus the ability of topoisomerase II to form a 'cleavable complex' in which the DNA is covalently linked to the enzyme.*

INTRODUCTION

IN RECENT YEARS, a new class of antitumour agent has been defined with the ability to induce DNA double-stranded breaks by stimulating the formation of a complex between the enzyme topoisomerase II (topo II) and DNA. Amsacrine, a clinical antitumour agent first synthesized by Cain and Atwell [1], was one of the first compounds found to have this action [2]. Other agents in this class include doxorubicin, daunorubicin and etoposide [3, 4] together with the acridine derivatives CI-921 [5] and acridine carboxamide [6] (see Fig. 1 for structures). Although the detailed sequence of events leading to cell death has not yet been elucidated [7], it is clear that the initial steps in drug action involve the sequence-selective DNA binding by the dimeric enzyme followed by the stabilization of covalent phosphotyrosine bonds linking the DNA to each enzyme monomer [8].

All of the above antitumour agents with the exception of etoposide bind to DNA by intercalation. However, intercalation is not sufficient for the action of topo II-directed cytotoxic agents since simple DNA intercalators such as 9-aminoacridine (9AA) and ethidium bromide do not stimulate the formation of the covalently bound complex and do not act through this enzyme to kill cells [6, 9]. They do, however, inhibit topo II-mediated unknotting or relaxation of closed circular duplex DNA, and can also protect against the action of a topo II-directed agent [9]. DNA-binding non-cytotoxic compounds which effectively protect against the cytotoxic action of topo II-directed drugs might be expected to have a number of applications. They could be used to chemoprotect a sensitive host tissue such as bone marrow against the effect of an accidental overdose of topo II-directed drug, or to rescue bone marrow cells following intentional high dose therapy. Furthermore, a chemoprotector with limited diffusion properties might be expected to have its highest concentration and thus its greatest

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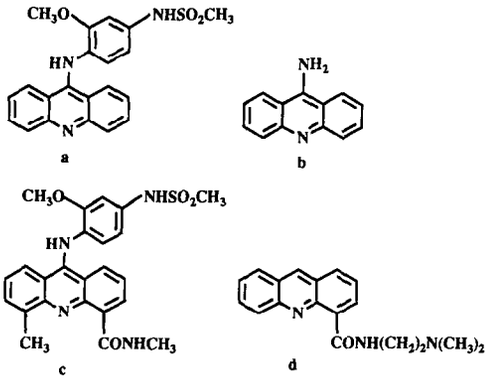

Fig. 1. Chemical structures (free base forms) of (a) amsacrine; (b) 9-aminoacridine; (c) CI-921 and (d) acridine carboxamide.

protecting activity in well vascularized normal tissues. Conversely, its absence in poorly perfused areas such as those existing in solid tumours would maximize tumour-selective cytotoxic effects. The latter principle has been proposed in designing radioprotectors which limit radiation-induced damage in well vascularized areas but not in poorly vascularized tumour tissue [10].

In this study we have compared the *in vitro* and *in vivo* action of three acridine derivatives as potential chemoprotectors against topo II-directed cytotoxic agents. 9AA (Fig. 1) is an antibacterial [11], *o*-AMSA is a isomer of amsacrine [12] and quinacrine is an antimalarial [11]. The effects of these compounds on the action of a number of topo II-directed agents have been evaluated using Lewis lung carcinoma and MM96 melanoma cells growing in culture, and against P388 leukaemia growing in mice. The topo II-directed agents comprise amsacrine, etoposide, mitoxantrone, doxorubicin, CI-921 and acridine carboxamide. CI-921 is an amsacrine analogue selected for high activity against the Lewis lung carcinoma [5] and currently in clinical trial while acridine carboxamide is a new acridine derivative with high activity against Lewis lung tumours [13].

MATERIALS AND METHODS

Drugs

Amsacrine isethionate, CI-921 isethionate and [^{14}C]CI-921 (labelled in the 4-carboxamido substituent, specific activity 7980 Bq/pmol) were kindly supplied by Drs L. M. Werbel and L. R. Whitfield, Parke-Davis Division of Warner-Lambert, Michigan, U.S.A. The radiochemical purity of [^{14}C]CI-921 (>95%) was determined by high performance liquid chromatography (HPLC), performed as described below for 9AA but using a Hewlett Packard 1040M diode array absorbance detector together with scintillation counting of the eluate. Acridine carboxamide and *o*-AMSA were synthesized in the Cancer Research Laboratory, Auckland. 9AA and quinacrine were from Sigma, U.S.A.

In vitro *determination of drug cytotoxicity*

A Lewis lung tissue culture (LLTC) line, developed from the Lewis lung tumour at the Southern Research Institute, Birmingham, U.S.A., was obtained from Dr R. C. Jackson, Parke-Davis Division of the Warner-Lambert Company, Ann Arbor, Michigan, U.S.A. LLTC cells were cultured in 25 cm^2 plastic flasks. Growth medium consisted of αMEM (Gibco) supplemented with foetal calf serum (FBS; 10% v/v; Gibco NZ Ltd) and antibiotics (penicillin 100 units/ml, streptomycin 100 μM/ml) [14]. MM96 human melanoma cells were obtained from Dr R. Whitehead (Ludwig Institute, Melbourne, Australia) and cultured with growth medium further supplemented with insulin (2 μg/ml) and hydrocortisone (200 ng/ml).

Cells for cytotoxicity assays were grown in cultures and then treated with trypsin (Difco; 0.1% in 0.134 M KCl, 0.015 M trisodium citrate). The cells were collected by centrifugation and exposed to cytotoxic drugs in plastic tubes (10^5 cells/ml) in a 37°C water bath for 1 h. Chemoprotectors were added 20 min before the cytotoxic drugs and were left in the tubes for the duration of drug exposure. Cells were then collected by centrifugation, washed twice, resuspended in growth medium and counted using a Coulter counter. To estimate the surviving clonogenic fraction, various dilutions of cells were plated in 60 mm dishes (5 ml GM/dish), and incubated at 37°C in a humidified atmosphere of 5% carbon dioxide in air [14]. After 9 days (LLTC) or 11 days (MM96), colonies were stained with methylene blue (Sigma) and those containing over 50 cells counted. The plating efficiency was 60–100%.

Measurement of cellular uptake of 9AA

LLTC cells were suspended in growth medium (10 ml) at 10^5 cells/ml at 37°C and exposed to 9AA for 10 or 100 min at a concentration of 5 μM. Cultures were centrifuged (2000 ***g***, 2 min) and samples of the supernatant (75 μl) deproteinized by addition of 19 vol. ice-cold acetonitrile. Cell pellets were lysed by addition of H_2O (0.1 ml) followed by acetonitrile (0.9 ml) to precipitate macromolecules. After centrifugation supernatants were evaporated to dryness under vacuum, redissolved in mobile phase and analysed for 9AA by reverse phase HPLC. The chromatographic system comprised a Waters refrigerated (2°C) WISP model 712 autosampler and MR600 solvent delivery system (flow rate 1.4 ml/min) with a C18 μbondapak column (8 × 100 mm). The aqueous component of the mobile phase was heptanesulphonic acid (7.5 mM), dibutylammonium phosphate (5 mM) and NaH_2PO_4 (10 mM), pH 3.5, to which methanol was added to 48% v/v. The eluate was monitored with an HP 1046 programmable fluorescence detector using zero order excitation and emission.

Data capture and integration of the 9AA peak (retention time 12.5 min) was undertaken with a Hewlett Packard 310 computer using HP 79996A software. No correction for extracellular fluid trapped in pellets was necessary because of the high cell uptake factor of 9AA.

Measurement of cellular uptake of [^{14}C]*CI-921*

Single cell suspensions of LLTC (2.1×10^6/ml) in αMEM containing 10% FBS and 20 mM hepes, pH 7.2, were incubated at 37°C with [^{14}C]CI-921 (0.6 μM) with or without chemoprotectors added at the same time as the labelled drug. After 60 min, samples (1 ml) were centrifuged (2000 ***g***, 2 min) and radioactivity in the supernatant was measured by scintillation counting. The remaining medium was aspirated and the centrifugation and aspiration repeated to minimize extracellular medium in the tubes. Pellets were solubilized in 0.1 ml Soluene 350 tissue solubilizer (Packard Instruments, Groningen, Netherlands) and counted in a water-accepting scintillation cocktail (Dimilume 30) in a Packard 1500 Tricarb liquid scintillation spectrometer.

Fluorescence microscopy

LLTC cells were grown on glass microscope cover slips in growth medium to half-confluence. 9AA (5 μM) was added and the cells were incubated at 37°C for 10 min. Cover slips were then inverted and placed in a live cell observation chamber in a Nikon Optiphot fluorescence microscope with a Model EF-D fluorescence attachment. Cells were illuminated with ultraviolet light (300–380 nm) and observed through a blue emission filter.

In vivo *determination of drug effects against P388 leukaemia*

P388 cells (originating from the National Cancer Institute, U.S.A.) were passaged i.p. in DBA/2J mice and grown for experiments by i.p. inoculation of 10^6 cells in DBA/2J male × C57BL/6J female hybrid mice [15]. Experiments consisted of 15–20 control mice with six mice per experimental group. Drugs were administered on days 1, 5 and 9 after tumour inoculation, and doses were calculated on the basis of the body weight at the time of the first injection. The mean survival time for control and treated groups was calculated and percentage increase in lifespan calculated as [100 × (treated/control −1)]. In toxicity experiments, non tumour-bearing animals were treated with the same dosage schedule and observed for 50 days thereafter.

RESULTS

Abrogation of in vitro *cytotoxicity of CI-921 by acridine derivatives*

To determine whether DNA intercalators with low cytotoxic potency protect against the cytotoxicity of a topo II-directed agent, LLTC cultures were exposed for 1 h to CI-291 with or without 9AA, *o*-AMSA or quinacrine at 20 μM (added 20 min beforehand). CI-921 reduced survival to 1–4% when used alone. 9AA and *o*-AMSA had little or no toxicity under these conditions, but completely prevented killing by CI-921 (Table 1). Quinacrine showed some toxicity at this concentration but also suppressed the toxicity of CI-921 (Table 1).

The dependence of the 9AA effect on the time of its addition is shown in Fig. 2. 9AA was most effective when added before or at the same time as CI-921 and showed minimal effect when added immediately before the cells were collected by centrifugation, washed and plated for assay of survival. The effect for intermediate times was directly proportional to the exposure period, suggesting that CI-921 killing was continuous during the 1 h exposure period, and the 9AA halted further killing rapidly after its addition.

Effect of acridine derivatives on cellular uptake of CI-921

The effect of 9AA, *o*-AMSA and quinacrine on the uptake of [^{14}C]CI-921 by LLTC cells was measured (Table 2). The mean volume of LLTC cells was estimated as 2.5 pl by analysis of Coulter pulse heights, suggesting a mean water content of approx. 2 pl/cell. On this basis the mean intracellular CI-921 concentration in untreated cells was estimated at 73.5 μM and extracellular concentrations were measured at 0.3 μM, indicating cell

Table 1. *Chemoprotection against CI-921 cytotoxicity by intercalating agents*

	Surviving fraction		
Chemoprotector	CI-921 only	Protector only	CI-921 + protector
9-AA	0.011 ± 0.005	0.59 ± 0.03	0.64 ± 0.08
Quinacrine	0.011 ± 0.005	0.09 ± 0.02	0.080 ± 0.004
o-AMSA	0.040 ± 0.005	0.82	1.07

LLTC cells were exposed to CI-921 (1 μM, 60 min) or to protector (20 μM, 80 min) or to both with protector added 20 min prior to CI-921. Values are mean ± S.E.M. for duplicate cultures.

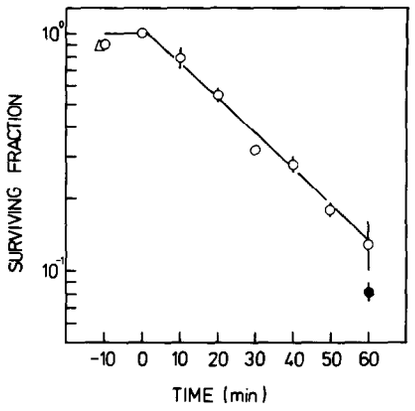

Fig. 2. Effect of addition of 9AA before or after CI-921. CI-921 was added to LLTC cells (final concentration 1 μM) at t = 0 and the incubation continued for 60 min. 9AA (final concentration 5 μM) was added to separate cultures at the times indicated on the abscissa, starting 10 min before CI-921 addition. Values represent mean surviving fractions ± S.E.M. of two independent experiments. (△) 9AA (5 μM) in the absence of CI-921; (●) CI-921 (1 μM) in the absence of 9AA.

Table 2. Cell-associated CI-921 after incubation of LLTC cells with [^{14}C]CI-921 (0.6 μM) in the presence or absence of chemoprotectors for 60 min

Protector	Concentration (μM)	Cell-associated CI-921 (pmol/10^6 cells)
—	—	147 ± 3*
9-AA	2	149 ± 2
	5	147 ± 2
	10	157 ± 5
	20	154 ± 5
o-AMSA	20	183 ± 5
Quinacrine	20	169 ± 3

*Values are mean ± S.E.M. for triplicate determinations. The control (no protector) value corresponded to 46,700 ± 800 dpm/pellet.

uptake factors (intracellular/extracellular concentrations) of approx. 250. 9AA had little effect on uptake while both *o*-AMSA and quinacrine slightly stimulated uptake.

Cellular uptake of 9AA

An HPLC assay with fluorimetric detection was used to investigate uptake of 9AA by LLTC cells. Recoveries of 81 ± 1 and 75 ± 2% (mean ± S.E.M., $n = 3$) respectively were obtained after spiking extracellular medium and cell pellets with 9AA. During incubation of LLTC cells with 9AA (5 μM) uptake was essentially complete by 10 min, the first time examined, with a small subsequent increase from 4.3 ± 0.3 to 5.2 ± 0.2 nmol 9AA/10^6 cells by 100 min. Extracellular concentrations were 3.3 ± 0.1 and 2.8 ± 0.2 μM at these two times. Average intracellular concentrations were calculated as above and indicated uptake factors of 650 and 930 at 10 and 100 min respectively.

The subcellular distribution of 9AA in LLTC cells was determined using coverslip cultures. As observed in fluorescence microscopy, 9AA was concentrated mainly in cytoplasmic vesicles although weak nuclear flourescence was also observed. By comparison with the fluorescence distribution of rhodamine 123 (which stains mitochondria) and acridine orange (which stains endosomes) it was clear that 9AA was accumulated by ctyoplasmic endosomes.

Effect of 9AA on cytotoxicity of other topo II-directed antitumour agents

LLTC cells were exposed to a single concentration (5 μM) of 9AA added 20 min before addition of different concentrations of cytotoxic drugs. Efficient protection against cytotoxicity was found with amsacrine, acridine carboxamide and etoposide, while little effect was found with mitoxantrone and none with doxorubicin (Fig. 3). The concentration dependence of the 9AA effect was also determined using these compounds. Again, little effect was observed with doxorubicin (Fig. 4).

The effect of 9AA was also measured using cultures of MM96 human melanoma cells. 9AA protected against the cytotoxicity of CI-921 but not that of doxorubicin (Fig. 5), even when cells were incubated with 9AA during the time of exposure to doxorubicin and for an additional 3 h after removing cells from doxorubicin.

Protection against in vivo *cytotoxicity by acridine derivatives*

Mice were inoculated i.p. with P388 leukaemia, then treated i.p. with CI-921 (5.9 mg/kg, administered on days 1, 5, 9). An average increase in lifespan of 74 (±10% S.D.) was obtained over four experiments. When injected i.p. with 9AA (20 mg/kg/dose), *o*-AMSA (45 mg/kg/dose) or quinacrine (45 mg/kg/dose) at the same time as CI-921, the increase in lifespan was 15%, 67% and 86% respectively. 9AA was therefore the most effective of the three compounds in reducing the *in vivo* antitumour effect of CI-921. Mice were also inoculated i.v. with P388 leukaemia and treated i.p. with CI-921 (5.9 mg/kg, days 1, 5, 9) with or without simultaneous i.p. injection of 9AA (20 mg/kg/dose), *o*-AMSA (45 mg/kg/dose) or quinacrine (45 mg/kg/dose). In this case no decrease in lifespan was induced by treatment with the chemoprotectors. Treatment of mice with P388 leukaemia with 9AA, *o*-AMSA or quinacrine alone at these doses (administered intraperitoneally on days 1, 5, 9) produced no significant life extension, while higher doses were toxic.

The *in vivo* effect of 9AA on the antitumour effects of several cytotoxic drugs was then compared by simultaneously administering them i.p. with 9AA

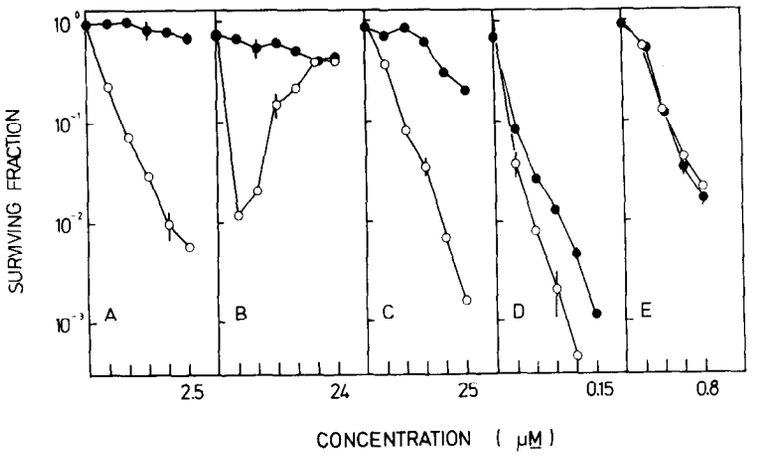

Fig. 3. Effect of 9AA (5 μM) on the cytotoxicity of antineoplastic agents interacting with topoisomerase II. Exponentially growing LLTC cells were exposed to antineoplastic drugs for 1 h in the absence (○) or presence (●) of 9AA. When present, 9AA was added 20 min before the antineoplastic drugs. Values represent means ± S.E. of duplicate cultures. A: CI-921; B: acridine carboxamide; C: etoposide; D: mitoxantrone; E: doxorubicin.

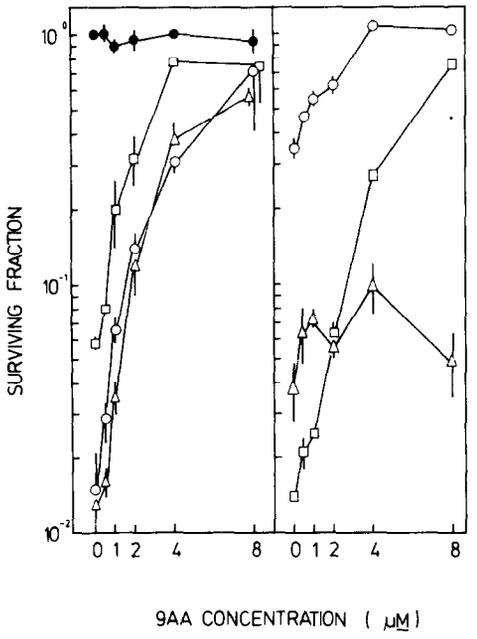

Fig. 4. Concentration dependence of the ability of 9AA to reverse cytotoxicity of antineoplastic agents interacting with topoisomerase II. Exponentially growing LLTC cells were exposed to 9AA (0–8 μM). After 20 min anticancer drugs were added and the incubation continued for 1 h. Values represent means ± S.E.M. of two independent experiments (left panel) or of duplicate cultures in single experiments (right panel). Left panel: (●) no addition to 9AA; (○) amsacrine, 4 μM; (□) CI-921, 1 μM; (△) acridine carboxamide, 5 μM. Right panel: (○) etoposide, 4 μM; (□) etoposide, 15 μM; (△) doxorubicin, 0.5 μM.

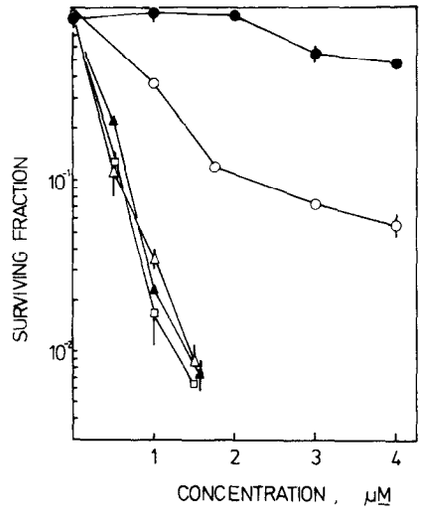

Fig. 5. Effect of 9AA on cytotoxicity of CI-921 and doxorubicin against exponentially growing human melanoma cells. MM96 cells were exposed to CI-921 for 1 h in the absence (○) or presence (●) of 9AA (5 μM), or to doxorubicin for 1 h in the absence (△) or presence of 9AA added 20 min before doxorubicin and removed at the same time as doxorubicin (▲) or added 20 min before doxorubicin and removed from 3 h after doxorubicin (□).

with the same intermittent dose schedule as above. A reduction of antitumour effect was noted with amsacrine, CI-921, acridine carboxamide and etoposide, and little or no effect was found with mitoxantrone and doxorubicin (Table 3).

Treatment of non-leukaemic mice with doses of amsacrine (20 mg/kg/dose) or CI-921 (45 mg/kg/dose) on an intermittent schedule (as above) was toxic (6/6 deaths; average times of death 8.2 and 13.0 days, respectively). Simultaneous administration of 9AA (13.3 mg/kg) protected fully against amsacrine toxicity (0/6 deaths) and partially against CI-921 toxicity (3/6 deaths, average time of death 11.3 days).

DISCUSSION

The results demonstrate that simple DNA intercalating agents, termed here chemoprotectors, can be used to inhibit the *in vitro* cytotoxic action of amsacrine, CI-921, acridine carboxamide and etoposide. It has previously been shown that ethidium inhibits topo II action in a cell free system and also hinders the cytotoxic action of etoposide in cultured cells [9]. Furthermore, the induction of DNA break-

Table 3. In vivo *effects of 9AA on antitumour drugs which target topoisomerase II*

Drug	Dose* (mg/kg/dose)	9AA dose (mg/kg/dose)	ILS† (%)
Amsacrine	5.9	0	60
		13.3	33
CI-921	5.9	0	74
		13.3	21
Acridine carboxamide	45	0	43
		13.3	17
Etoposide	5.9	0	89
		13.3	43
Mitoxantrone	2.6	0	96
		13.3	75
Doxorubicin	3.9	0	67
		13.3	75

*Administered on days 1, 5, 9 after tumour inoculation.
†Increase in lifespan with respect to untreated animals.

age by amsacrine and etoposide is inhibited by a bis-intercalating drug ditercalinium [16], and that by etoposide is inhibited by doxorubicin [17].

The results also show that the *in vivo* antitumour effect of amsacrine, CI-921, acridine carboxamide and etoposide can be reduced or abolished by co-administration of 9AA, and that host toxicity of amsacrine and CI-921 can also be reduced. A significant reduction in antitumour effect is observed only when both P388 cells and drugs are administered i.p. The lack of effect of 9AA against i.v. implanted tumour cells suggests that 9AA does not distribute well from its site of injection. It may be possible to design chemoprotectors with better distribution properties, using the same rationale as was used in the design of amsacrine analogues for activity against tumours implanted remotely from the site of drug injection [5].

There are several possible mechanisms by which 9AA may protect against topo II-mediated drug cytotoxicity. One is that the chemoprotectors might act through the inhibition of uptake of CI-921. The results shown in Table 2 indicate on the contrary that 9AA, *o*-AMSA and quinacrine, at concentrations sufficient to cause full protection against CI-921 toxicity, do not inhibit CI-921 uptake. In fact, a slight stimulation of uptake is evident, possibly reflecting the inhibition of CI-921 efflux.

A second possibility is that the chemoprotector competitively inhibits the binding of the topo II inhibitor to DNA. This is unlikely for four reasons.

(a) Etoposide is thought to bind primarily to the topo II enzyme rather than to DNA [4], yet its toxicity is effectively countered by 9AA.

(b) Acridine carboxamide inhibits its own cytotoxic action at supra-optimal concentrations ([14] and Fig. 3B). This suggests that at high DNA binding ratios it chemoprotects in the same way as does 9AA.

(c) If concentrations of chemoprotectors in the nucleus are sufficient to inhibit competitively the DNA binding of CI-921, a decrease in total cell uptake of [^{14}C]CI-921 might be expected. This is not observed (Table 2).

(d) The amount of 9AA associated with the DNA under the conditions of the experiment may be insufficient to displace the topo II inhibitor from the DNA. The uptake studies show, under conditions providing nearly maximal protection (5 μM 9AA), that sufficient drug is available (approx. 5.2 nmol/10^6 cells) for a maximum drug:DNA base pair binding ratio of 0.28 (assuming a DNA content in these aneuploid cells of 10 pg/cell). Since 9AA will bind to RNA and protein, and since fluorescence microscopy indicates that a substantial fraction of visible cell-associated 9AA is sequestered in cytoplasmic vesicles, the intracellular DNA binding ratio is likely to be much lower than 0.28 and is thus probably too low to effectively compete with the binding of other agents.

A third possible mechanism is that the chemoprotector inhibits the formation of the 'cleavable complex' form of topo II. Simple DNA intercalators are well known inhibitors of topo II-mediated DNA strand passing reactions [6, 9], consistent with inhibition of 'cleavable complex' formation. The formation of the 'cleavable complex' involves a conformational change in the DNA, and it is possible that if DNA around the topo II binding site is altered by drug intercalation, its ability to undergo conformational changes will be reduced. The time course of addition of 9AA (Fig. 2) is consistent with the hypothesis that the addition of 9AA causes the reversal of formation of the 'cleavable complex', thus preventing further cell death.

A fourth possible mechanism is that 9AA affects steps subsequent to formation of the topo II 'cleavable complex'. Although the cytotoxicity of amsacrine and etoposide is related to the extent and duration of formation of the 'cleavable complex', the relationship between the induction of this complex and cell death is not understood [7]. 9AA may inhibit one or more of the steps in this process. This hypothesis would also be compatible with the time course experiments shown in Fig. 2.

The relative lack of effect of 9AA on the cytotoxicity of doxorubicin and mitoxantrone is noteworthy. The activity of the latter agents is dependent on the presence of topo II since an alteration in resistant cells [18] or a decrease in the activity of this enzyme in non-cycling cells [19, 20] is associated with resistance. On the other hand, doxorubicin does not stimulate the formation of DNA–protein cross-links to the extent demonstrated for amsacrine [21]. One possible explanation for these divergent results is that doxorubicin and mitoxantrone require the presence of topo II but act by a different mechanism which is less sensitive to 9AA and related agents.

In conclusion, the modulation of cytotoxic activity of drugs such as etoposide and amsacrine not only raises questions about mechanism, but also suggests possibilities for therapeutic application. It is noteworthy that, at least in culture, 9AA can reduce toxicity even when given some time after the addition of a cytotoxic agent. If this principle also applies to *in vivo* treatment it would be useful in cases of accidental drug overdose. The three chemoprotectors identified here, 9AA, *o*-AMSA and quinacrine, have the disadvantage of being rather toxic to the mouse, but it may be possible to develop chemoprotectors with lower intrinisic toxicity and greater activity for clinical use. If these compounds have less efficient tissue distribution properties than the cytotoxic agent, they might be used to spare rapidly growing and highly vascularized normal tissues such as bone marrow from cytotoxic effects.

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