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INDUCTION OF DNA–PROTEIN CROSSLINKS BY ANTITUMOR 1-NITRO-9-AMINOACRIDINES IN L1210 LEUKEMIA CELLS

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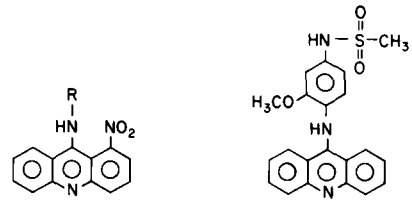
Abstract—Ledakrin [1-nitro-9-(3′-dimethylamino-*N*-propylamino)acridine], an antitumor drug of the 1-nitro-9-aminoacridine family, was able to induce DNA-protein crosslinks in intact L1210 leukemia cells, as demonstrated by the potassium–dodecyl sulfate precipitation technique. Ledakrin-induced DNA–protein crosslinks were not readily reversible nor were they accompanied by DNA double-strand breaks. Also, ledakrin produced virtually no crosslinks in isolated nuclei. Ledakrin-induced DNA–protein crosslinks seemed not to be mediated by topoisomerase II, unlike well-established effects of a chemically related antitumor drug, 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (*m*-AMSA). Four ledakrin analogs of divergent cytotoxic potencies also induced DNA–protein crosslinks but not DNA double-strand breaks in intact L1210 cells. A significant positive correlation existed between the ability of ledakrin and its 1-nitro analogs to induce DNA–protein crosslinks and the antiproliferative effects of these drugs. The results are consistent with the previously shown ability of 1-nitro-9-aminoacridines to covalently bind to macromolecules after metabolic activation in the cell. In addition to previously demonstrated DNA interstrand crosslinks and monofunctional adducts, DNA–protein crosslinks constitute another type of DNA lesion induced by 1-nitro-9-aminoacridines.

A large number of 1-nitro-9-aminoacridine derivatives, including the clinically used drug ledakrin (also known as nitracrine), exhibit potent cytotoxic and antitumor properties [1–5]. In the cell, these drugs undergo metabolic activation, a prerequisite for covalent binding to DNA and other cellular macromolecules [4–6]. In addition to monofunctional adducts, ledakrin and its analogs are capable of bifunctional binding inducing DNA interstrand crosslinks [3, 7]. Significant, positive correlations exist between both types of DNA lesions and the cytotoxicity of 1-nitroacridines [7, 8]. DNA damage induced by ledakrin impairs DNA template properties leading to inhibition of DNA replication [9], RNA synthesis [2, 10] and, eventually, a block in S phase of the cell cycle [11].

m-AMSA,‖ another antitumor derivative of 9-aminoacridine, has received attention recently because of its ability to induce protein-associated DNA damage mediated by topoisomerase II. This enzyme alters DNA topology by passing duplex DNA through a transient double-strand break [12, 13]. *m*-AMSA stabilizes an intermediate of this reaction, the "cleavable complex," characterized by DNA double-strand breaks with the enzyme bound covalently to the ends of the broken strands [12–15]. The enzyme-mediated lesions, DNA–protein crosslinks and DNA breaks, are believed to play a crucial role in the antitumor properties of *m*-AMSA and other various intercalating drugs [12–15].



Compound	R	m-AMSA
Ledakrin	$-(CH_2)_3N(CH_3)_2$	
C-609	$-(CH_2)_4N(CH_3)_2$	
C-857	$-(CH_2)_2OH$	
C-835	$-(CH_2)_2N(CH_2CH_2OH)_2$	
C-1006	$-(CH_2)_3NH(CH_2)_5CH_3$	

Fig. 1. Structures of ledakrin and related 1-nitro-9-aminoacridines and *m*-AMSA.

It is unclear which features of 9-aminoacridines are needed for the interference with topoisomerase II. The structures of ledakrin and *m*-AMSA share the 9-aminoacridine core (Fig. 1). In addition, ledakrin is a more efficient DNA intercalator [2] than *m*-AMSA [16]. It was not known whether ledakrin and related 1-nitro-9-aminoacridines also shared with *m*-AMSA the ability to induce topoisomerase II-mediated

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‖ Abbreviations: *m*-AMSA, 4′-(9′-acridinylamino) methanesulfon-*m*-anisidide; C_{50}, drug concentration required for covalent crosslinking of protein to 50% of total DNA in the sample; IC_{50}, drug concentration required to inhibit cell growth by 50% after 48 hr; L1210, leukemia L1210 cells; and SDS, sodium dodecyl sulfate.

DNA damage. On the other hand, the known ability of ledakrin to induce DNA–DNA crosslinks in intact cells suggested that analogous bifunctional covalent binding of the drug might occur between DNA and nuclear proteins.

This study determined the ability of ledakrin and its analogs to induce protein-associated lesions. The nature of this damage was evaluated and its relationship to cytotoxicity assessed. The results indicate that ledakrin and its congeners induce DNA–protein crosslinks. Unlike *m*-AMSA, ledakrin-induced lesions seem not to be mediated by topoisomerase II. These lesions probably reflect covalent binding of the drug to macromolecules. DNA–protein crosslinks are a new type of DNA damage induced by 1-nitro-9-aminoacridines.

MATERIALS AND METHODS

Drugs. Ledakrin [1-nitro-9-(3′-dimethylamino-*N*-propylamino)acridine] and other 1-nitro-9-aminoacridines differing in the side chain (Fig. 1), as well as compound C-264 [2-nitro-9-(3′-dimethylamino-*N*-propylamino)acridine; structure not shown], were provided by the late Professor Andrzej Ledochowski from the Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdansk, Poland. Novobiocin was obtained from Sigma, while *m*-AMSA [4′-(9-acridinylamino)methanesulfon-*m*-anisidide; Fig. 1)] was supplied by the Warner–Lambert Pharmaceutical Co. Nitroacridines and novobiocin were dissolved in water and *m*-AMSA in dimethyl sulfoxide. Drug stock solutions were stored frozen in the dark.

Tissue culture and cytotoxic activities. Leukemia L1210 cells were cultivated in RPMI 1640 medium supplemented with 10% calf serum and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes). Cytotoxic activities of these drugs against leukemia L1210 cells (IC_{50}: drug concentrations required to inhibit cell growth by 50%) were determined as described previously [17]. In this assay, the cells at an initial concentration of 2–4 × 10^4/ml were exposed continuously to drugs for 48 hr.

DNA–protein crosslinks in intact cells. Cells in the exponential phase of growth were labeled for 18–40 hr with [2-^{14}C]thymidine (0.06 to 0.1 μCi/ml, 56 Ci/mmol, Moravek Biochemicals, Brea, CA, U.S.A.) The cells were resuspended at 10^6/ml in fresh medium without [^{14}C]thymidine and incubated for 30 min prior to drug treatment at 37°. After incubation with drug, cells were pelletted (280 *g*, 7 min) and resuspended in phosphate-buffered saline. The level of DNA covalently bound to protein was determined by the potassium dodecyl sulfate coprecipitation assay of Rowe *et al.* [15]. Cell suspensions were mixed with an equal volume of lysing solution (2.5% SDS, 10 mM EDTA, 0.4 mg/ml salmon sperm DNA, pH 8.0) followed by incubation for 15 min at 65°. At the end of the incubations, samples were vortexed for 15 sec and supplemented with KCl (65 mM final concentration). After 15 min on ice, the precipitate was collected by centrifugation (2000 rpm for 15 min at 4°) and redissolved in washing solution (10 mM Tris, pH 8.0, 1 mM. EDTA, 0.1 μg/ml salmon sperm DNA) by heating to 65°. After reprecipitation on ice the pellets were dissolved, precipitated again, and hydrolyzed in 0.5 ml of 2 M perchloric acid for 1 hr at 70° prior to determination of radioactivity by liquid scintillation counting. Separate aliquots of cells (10^6) in phosphate-buffered saline were hydrolyzed in perchloric acid and used for the determination of total radioactivity. The amount of DNA co-precipitable with protein is expressed as percent of total DNA.

DNA–protein crosslinks in nuclei. Nuclei from L1210 cells were isolated under isotonic conditions according to Glisson *et al.* [18]. Isolated nuclei were resuspended in the isolation buffer [2 mM KH_2PO_4, 5 mM $MgCl_2$, 150 mM NaCl, 1 mM ethylenebis(oxyethylenenitrylo)tetraacetic acid (EGTA), pH 6.9] with or without 0.4 mM ATP at 0.3–0.5 × 10^6 nuclei/ml. Drug treatment of nuclei was carried out at 37° for 90 min. Subsequently, the samples were mixed with an equal volume of lysing solution and analyzed for DNA–protein crosslinks as described for intact cells.

Filter elutions. Cells for filter elution were labeled with [^{14}C]thymidine and treated with drugs as described for the determination of DNA–protein crosslinks. Alkaline filter elution was carried out as described by Kohn *et al.* [19]. The cells were either applied onto polyvinyl chloride filters (Millipore, pore size 2 μm), lysed without proteinase K and eluted with 2% tetrapropyl ammonium hydroxide, 10 mM EDTA, pH 12.1, or on polycarbonate filters (Nucleopore, pore size 0.8 μm), lysed with proteinase K (0.5 mg/ml) and eluted with 0.1% SDS, 2% tetrapropyl ammonium hydroxide, 10 mM EDTA, pH 12.1.

The filter elution procedure for the determination of DNA double-strand breaks [20] was used as described elsewhere [21]. Cells treated with drugs (around 0.3 × 10^6) were lysed onto polycarbonate filters with SDS and proteinase K (1 hr at 37° followed by 30 min at 20°). The elution was carried out with 2% tetrapropyl ammonium hydroxide, 0.2% SDS, 20 mM EDTA, pH 9.7.

RESULTS

The involvement of proteins in DNA lesions induced by antitumor drugs can be assessed by comparing profiles of alkaline filter elution either with or without proteinase K digestion. Initially, we used this approach to characterize DNA lesions induced in L1210 cells by ledakrin. The lysis in the presence of proteinase K revealed DNA fragmentation in cells treated with ledakrin (at 2 and 5 μM) as indicated by a substantially enhanced elution of DNA compared to untreated control (Fig. 2). These data were consistent with previous findings by sucrose gradient sedimentation [8]. In contrast, virtually no cleavage was detected with 2 or 5 μM ledakrin when the cells were lysed in the absence of proteinase K as evidenced by negligible amounts of eluted DNA (Fig. 2). These observations suggested that ledakrin-induced DNA breaks were accompanied by DNA–protein crosslinks.

There is strong evidence that ledakrin induces other types of DNA lesions such as monofunctional adducts and interstrand crosslinks [3–7] in addition to

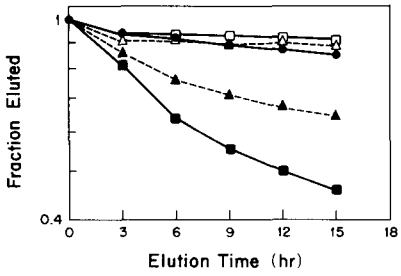

Fig. 2. Alakaline elution profiles of DNA from L1210 cells incubated without drug (control, ●) and with ledakrin at 2 μM (▲, △) or 5 μM (■, □) for 60 min at 37°. The cells were lysed in the presence (solid symbols) or absence (open symbols) of proteinase K as described in Materials and Methods. The elution profile for control cells in the absence of proteinase K (omitted for the sake of clarity) was very close to the profile for 2 μM ledakrin in the absence of proteinase K.

DNA cleavage and DNA–protein crosslinks. DNA alkaline elution patterns could then reflect overlapping of these effects. Additionally, some of these lesions may be alkali labile [8]. Another technique based on co-precipitation of DNA with protein–potassium dodecyl sulfate complexes selectively determines only covalent DNA–protein crosslinks. This assay (done at neutral pH) was utilized to demonstrate and characterize the ability of ledakrin to induce DNA–protein crosslinks. The results in Fig. 3A show that incubation of L1210 cells with ledakrin (2–50 μM) resulted in a concentration-dependent increase in the fraction of DNA which co-precipitated with proteins. While ledakrin at 50 μM caused the precipitation of approximately 70% of total DNA, the effect did not reach a saturation level. Thus, ledakrin is a potent inducer of covalent DNA–protein crosslinks in intact L1210 cells. Under the same conditions, formaldehyde, used as a model agent capable of non-specific covalent linking of proteins to DNA, produced a detectable effect at 200 μM and approximately 80% of crosslinked DNA at 1 mM (data not shown).

Ledakrin effects were further characterized with regard to their kinetics of formation and rate of reversal. DNA–protein crosslinks generated by ledakrin (at 10 μM) increased continuously for at least 90 min (Fig. 3B). Furthermore, when cells pretreated with ledakrin (10 μM for 90 min) were incubated for up to 3 hr in a drug-free medium, the level of DNA–protein crosslinks did not change markedly (Fig. 3B). Even after 24 hr of post-treatment incubation, a substantial fraction of DNA (28% of total DNA) remained crosslinked to protein (not shown).

There was a possibility that, like for the related drug, *m*-AMSA, ledakrin-induced DNA–protein crosslinks might reflect stabilization of cleavable complexes of topoisomerase II. However, *m*-AMSA-induced crosslinks occurred faster than those of ledakrin, reaching a plateau at 10 min ([22] and data not shown). Moreover, topoisomerase II-mediated lesions, in general, are readily reversible [12–15]. Most of the *m*-AMSA-induced DNA–protein crosslinks disappeared after 30 min of incubation in fresh medium ([23] and data not shown), in contrast to the poorly reversible effects of ledakrin. Also, the *m*-AMSA effect shows a plateau at a drug concentration of 5–10 μM [24], whereas ledakrin-induced lesions continued to increase up to a ledakrin concentration as high as 50 μM.

To obtain clearer evidence that ledakrin-induced DNA–protein crosslinks are different from the lesions induced by *m*-AMSA, we compared both drugs in the context of known essential features of topoisomerase II-mediated effects of intercalating drugs. In particular, we determined whether ledakrin could induce (i) DNA–protein crosslinks in isolated nuclei and (ii) DNA double-strand breaks in whole cells.

Intact nuclei contain topoisomerase II which is functional in the presence of ATP [14]. Consistent with previous reports [14], *m*-AMSA retained its ability to induce DNA–protein crosslinks in nuclei

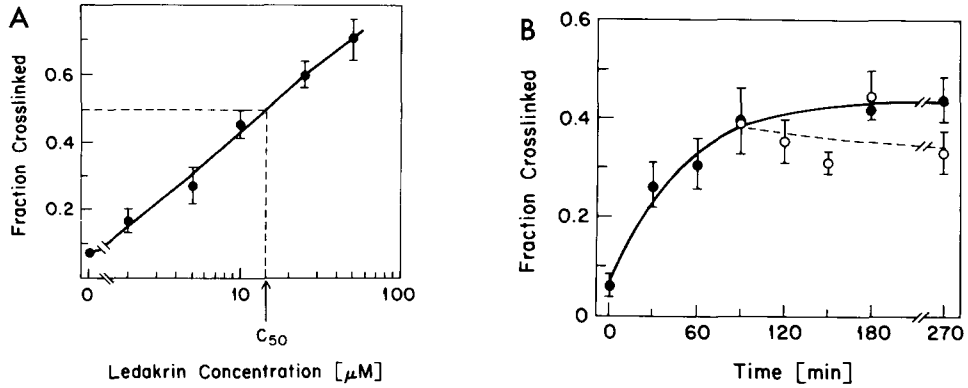

Fig. 3. DNA–protein crosslinks induced by ledakrin in intact L1210 cells. (A) Effects of various drug concentrations after a 90-min incubation at 37°. The dotted lines indicate drug concentration crosslinking 50% of DNA to protein (C_{50}). (B) Time-course of the effect of ledakrin at 10 μM (—●—). The dashed line (—○—) shows DNA–protein crosslinks after resuspending the cells treated with drug for 90 min in a fresh medium without ledakrin followed by further incubation at 37°. The data are mean values (± SE) from two to twelve determinations carried out in duplicate.

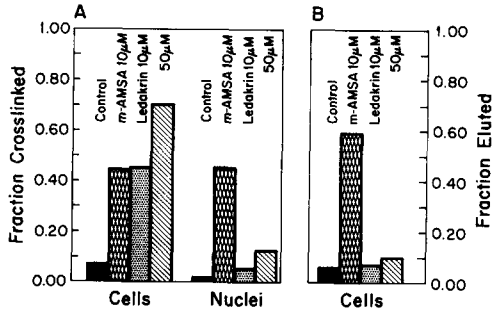

Fig. 4. Comparison of DNA-lesions induced by ledakrin and *m*-AMSA in intact L1210 cells and nuclei. Samples of L1210 cells or nuclei were incubated with ledakrin for 90 min or with *m*-AMSA for 30 min at 37° followed by determination of DNA–protein crosslinks (A) or DNA double-strand breaks (B) as described in Materials and Methods. The values of fraction-eluted DNA (B) represent data after 55 min of elution.

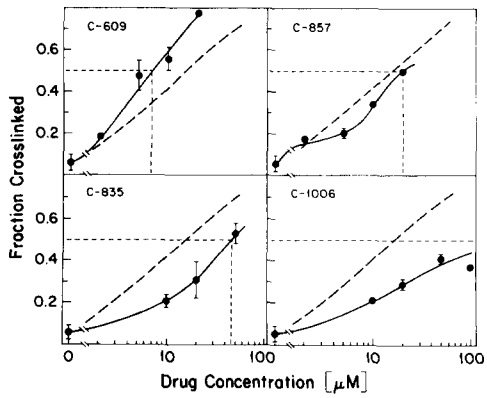

Fig. 5. Induction of DNA–protein crosslinks by ledakrin analogs (compounds C-609, C-857, C-835 and C-1006) in intact L121u cells after a 90-min incubation at 37°. The dotted lines indicate drug concentrations crosslinking 50% of DNA to protein (C_{50}). The data are mean values (± SE) from two determinations carried out in duplicate, except for the few points without error bars which are from a single experiment. The dashed line is a tracing of a crosslinking profile for ledakrin from Fig. 3A.

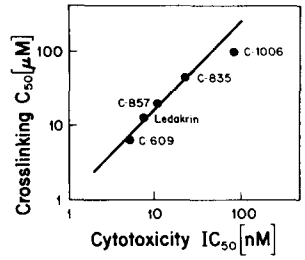

Fig. 6. Relationship between the ability of 1-nitro-9-aminoacridines to induce DNA–protein crosslinks in intact L1210 cells after 90-min treatment (C_{50}, determined from the data in Fig. 5) and their growth inhibitory potency (IC_{50}) after 48-hr treatment. The straight line shown was found by least square analysis and corresponds to the equation: log C_{50} [μM] = 0.957 × log IC_{50} [nM] + 0.246, with correlation coefficient, $r = 0.978$.

with 0.4 mM ATP (Fig. 4A). However, ledakrin was marginally active in nuclei under these conditions (Fig. 4A). The omission of ATP did not affect the results for ledakrin, but it prevented the effect of *m*-AMSA (data not shown).

The induction of DNA double-strand breaks, in addition to topoisomerase II-mediated DNA–protein crosslinks, is implied by the very nature of the cleavable complexes [12–15]. Accordingly, crosslinks induced by *m*-AMSA (10 μM) in whole cells were accompanied by DNA double-strand breaks (Fig. 4B). In contrast, ledakrin failed to produce this effect. Virtually no double-stranded cleavage was observed for ledakrin at concentrations of 10 or 50 μM (Fig. 4B). However, ledakrin at these same concentrations induced DNA–protein crosslinks at similar or substantially higher levels than *m*-AMSA at 10 μM (Fig. 4A).

DNA single-stranded cleavage induced by ledakrin (a consequence of the covalent binding of the drug to DNA) is reduced by novobiocin, an ATP antagonist [8]. Addition of 2 mM novobiocin also reduced DNA–protein crosslinks induced by ledakrin in whole cells by 66 or 52% for drug at 10 or 50 μM respectively. Although novobiocin is also a topoisomerase II inhibitor, it failed to affect *m*-AMSA-induced crosslinks in intact cells (data not shown) in accordance with the report by Pommier *et al.* [25].

To see if the ability to induce DNA–protein crosslinks in intact L1210 cells is a common feature of 1-nitro-9-aminoacridines, the effects of ledakrin were compared with those of four other derivatives: C-609, C-857, C-835, and C-1006 (for structures see Fig. 1). Figure 5 shows that all four ledakrin congeners were potent inducers of DNA–protein crosslinks. They differed markedly, however, in the concentrations required for this effect. C-609 was the most potent agent, while C-835 and C-1006 were the least active. C-857 showed an intermediate activity comparable to ledakrin. Like ledakrin, these four analogs failed to induce any significant double-stranded cleavage (data not shown).

To determine whether DNA–protein crosslinks might contribute to the antiproliferative effects of 1-nitro-9-aminoacridines, we examined the cytotoxicity of these drugs in L1210 cells and compared these activities with the ability of the drugs to induce DNA-protein crosslinks. In agreement with previous studies [7], the selected agents showed divergent cytotoxic activities (expressed as drug concentration inhibiting cell growth by 50%, IC_{50}; Fig. 6). While all of these compounds were very cytotoxic, the most active was derivative C-609 (IC_{50} = 5.2 nM) and the least active derivative C-1006 (IC_{50} = 82 nM). Intermediate IC_{50} values were found for ledakrin, C-857, and C-835 (7.6, 11.0, and 22.4 nM respectively).

The IC_{50} values for nitroacridines (as well as for *m*-AMSA) were substantially lower than drug concentrations inducing DNA–protein crosslinks. An analogous difference exists for *m*-AMSA. The latter drug was reported to exhibit a similar low IC_{50} value against L1210 cells (35 nM) [26] while inducing DNA damage at micromolar concentrations [15, 22, 23, 27] as in our study. Similar differences have also been

observed for other types of DNA lesions induced by nitroacridines [2–8]. These differences can be attributed to incomparable drug treatment conditions in both assays (cf. Materials and Methods). Nonetheless, the order of cytotoxic activities was identical as that seen for the crosslinking ability of the drug (expressed as the concentrations required to precipitate 50% of DNA; C_{50}). Figure 6 indicates the existence of a strong, statistically significant positive correlation between the C_{50} and IC_{50} values.

DISCUSSION

This study demonstrated the ability of ledakrin and other related 1-nitro-9-aminoacridine derivatives to induce covalent DNA–protein crosslinks in intact cells. This is a new type of DNA damage induced by these drugs in addition to previously characterized covalent monofunctional binding to DNA, interstrand crosslinking, single-strand breakage and generation of alkali labile sites [2–8].

Ledakrin and its analogs are known to bind covalently to DNA after metabolic activation in the cell [4, 5, 7, 8]. The pattern of ledakrin-induced DNA–protein crosslinking was fully consistent with this mechanism of action, and specifically with the bifunctional covalent binding of the drug to macromolecules. Covalent binding to cellular DNA, as well as interstrand crosslinking, require the nitro group in position 1 [5, 6]. This study showed that five 1-nitro-9-aminoacridines were able to induce DNA–protein crosslinks in L1210 cells. As found previously, 2-nitro-9-aminoacridines intercalate but do not bind covalently to DNA [6] and are several hundred-fold less cytotoxic than the 1-nitro isomers [2]. Accordingly, compound C-264, the 2-nitro isomer of ledakrin, failed to produce any crosslinking effect even at a very high concentration (100 μM, data not shown).

The level of ledakrin-induced DNA–protein crosslinks was reduced by the addition of novobiocin, an ATP antagonist. Previously, we observed that novobiocin diminishes the ability of ledakrin to generate alkali-labile sites and single-strand breaks in cellular DNA [8]. These inhibitory effects of novobiocin suggest that ATP may be involved in the metabolic activation of ledakrin and/or its covalent binding to macromolecules.

The DNA–protein crosslinks induced by ledakrin were probably not mediated by topoisomerase II, unlike the lesions caused by the related 9-aminoacridine drug, *m*-AMSA. The latter agent traps topoisomerase II bound to DNA in the enzyme intermediates (cleavable complexes). Stabilization of the cleavable complex, by definition, should result in DNA–protein crosslinks accompanied by an equivalent (or at least considerable) amount of double-strand breaks [12, 14, 21]. The results in this study were obtained in a side-by-side comparison with *m*-AMSA which served as a positive control and for which we detected a substantial amount of double-stranded cleavage. In contrast, ledakrin did not induce DNA double-stranded cleavage in whole cells. The lack of effect even with very high ledakrin concentrations is a qualitative difference compared to *m*-AMSA. Moreover, it is a well established fact for *m*-AMSA and other "classical" topoisomerase-targeted drugs that their cytotoxic activity is correlated with double-stranded cleavage [27]. Since the cytotoxic activity of ledakrin in L1210 cells (IC_{50} = 7.6 nM) was similar to that of *m*-AMSA (IC_{50} = 35 nM) [26], the lack of comparable double-stranded cleavage indicates that the mechanism of cytotoxicity of both drugs differs.

Ledakrin- and *m*-AMSA-induced DNA–protein crosslinks differ in several other respects. Unlike *m*-AMSA, ledakrin was unable to induce DNA–protein crosslinks in isolated nuclei, under the conditions where nuclear topoisomerase II remained functional. Thus, in contrast to *m*-AMSA, ledakrin (at least in its parental form) is unable to stabilize cleavable complexes. The negligible effect of ledakrin in isolated nuclei can be explained by a lack of drug metabolic activation in this system. While the activation of ledakrin can be mimicked in subcellular systems with the mediation of cytochrome P-450 [4], it is unlikely to occur without appropriate cofactors and cytoplasmic components absent from nuclei. We have also observed a lack of inhibition of DNA replication when drug was added to nuclei, whereas very potent inhibition was observed after drug treatment of whole cells [9]. It should be noted that activation is not required for ledakrin's ability to intercalate into DNA [2].

Furthermore, ledakrin-induced lesions showed a considerably slower rate of formation and poor reversibility as compared to the rapid formation and reversal characteristic of topoisomerase II-mediated DNA lesions [12, 14]. Slow kinetics of ledakrin-induced DNA–protein crosslinks parallels the time required for drug covalent binding to macromolecules [4, 5]. Although DNA–protein crosslinks caused by ledakrin are poorly reversible, their partial removal is consistent with the previously observed induction of DNA-repair synthesis [8]. Slow removal of the damage is also typical of covalent adducts [28].

Intracellularly activated drug (i.e. capable of bifunctional covalent binding to macromolecules [3, 4]) may crosslink various nuclear proteins to DNA, including topoisomerase II which is a major component of nuclear matrix [29]. Such a non-specific linkage need not be accompanied by strand breakage and should not be confused with topoisomerase-specific effects of *m*-AMSA and other "classical" topoisomerase-targeted drugs. On the other hand, it is unlikely that such an activated drug would specifically interact with the intermediate of the topoisomerase II reaction as does *m*AMSA or VM-26 especially when, as discussed, no double-stranded cleavage (which would result from specific interaction) was observed.

It is possible that ledakrin may interfere with the catalytic (strand passing) activity of topoisomerase II in a manner similar to *o*-AMSA or ethidium bromide [30, 31]. This was suggested by our observations that the 2-nitro isomer of ledakrin, DNA intercalator C-264 (which was unable to induce DNA–protein crosslinks itself), inhibited decatenation activity of isolated topoisomerase II and interfered with the stabilization of the cleavable complexes induced by VM-26 in nuclei and whole cells (data not shown). These effects, however, are typical of many inter-

calators and are unlikely to contribute significantly to the cytotoxic effects of 1-nitroacridines.

Not all of the analogs of *m*-AMSA trap cleavable complexes of topoisomerase II. For example, *o*-AMSA is unable to induce DNA–protein crosslinks [12, 13]. However, the results for ledakrin and its congeners are the first evidence that acridine drugs closely related to *m*-AMSA retain the ability to induce DNA–protein crosslinks but without stabilization of the topoisomerase II cleavable complex. It is also possible that topoisomerase-mediated DNA lesions may not represent the only mechanism of action for other drugs related to *m*-AMSA.

DNA lesions related to the covalent binding of 1-nitro-9-aminoacridines to DNA (i.e. DNA-interstrand crosslinks, alkali-labile sites, and single-strand breaks) have been shown previously to correlate with the antiproliferative activity of these drugs [7, 8]. This study demonstrates that a similar significant positive correlation exists for DNA–protein crosslinks induced by ledakrin and its four congeners. Thus, the latter DNA lesion seems to reflect the biological potential of 1-nitro-9-aminoacridines. As proposed previously, DNA-interstrand crosslinks may represent an important type of lesion induced by ledakrin and other 1-nitro-9-aminoacridines [3, 7]. DNA–protein crosslinks are likely to appear at higher frequency. Since the latter lesion is not readily reversible, it may contribute substantially to the impairment of DNA template properties [9, 10]. Further studies are needed to clarify the role of DNA–protein crosslinks in cell growth inhibition by nitroacridines.

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