Anti-Cancer Drugs 1999, **10**, pp. 163–170

Research paper

Intracellular localization of 6- and 7-substituted 2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3H-dibenz [*de,h*]isoquinoline-1,3-diones (azonafides) is not the limiting factor for their cytotoxicity: an *in vitro* confocal microscopy study

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The intracellular localization of 14 structurally unique azonafide analogs was studied to determine if intracellular drug distribution is the limiting factor in azonafide cytotoxicity. Using scanning laser confocal microscopy, cytotoxicity of the azonafide analogs studies was observed in Chinese hamster ovary cells immediately after a 1 h exposure. The intracellular drug distribution patterns varied significantly for different analogs. Eight analogs showed primarily nuclear localization, five analogs showed primarily cytoplasmic localization and two analogs displayed perinuclear localization. In general, the type of chemical substitution on the anthracene nucleus determined the distribution pattern. For example, for each analog seven of eight nuclear-localizing analogs were amine-substituted agents, while four of five cytoplasmic-localized agents were ethoxy-substituted analogs. The individual exception within these groups was the 6-[(dimethylamino)ethoxy] agent that was nuclear localized. The two perinuclear-localized agents included the unsubstituted parent, azonafide, and its 6-methyl azonafide analog. Comparison of the cytotoxicity of the azonafides, based on intracellular localization, revealed that none of the localization patterns were associated with increased cytotoxicity. These results show that minor structural changes in the azonafide class of antitumor agents involving substitution along an anthracene chromophore result in substantially different intracellular drug distribution patterns. However, these distribution differences do not determine relative cytotoxic potency *in vitro*. [© **1999 Lippincott Williams & Wilkins.**]

Key words: Anthracenes, distribution, intercalators, new agents.

Introduction

Several groups have used confocal microscopy to study the intracellular distribution of fluorescent anticancer drugs such as doxorubicin (DOX),[1] daunorubicin (DN)[1] and mitoxantrone (MTZ).[2] The assumption in these studies is that intracellular drug fluorescence reflects the true intracellular location of drug molecules, which has been documented to be accurate for DOX[1] and MTZ.[2] The intracellular distribution patterns of these two compounds are conserved between independent investigations where one group of investigators studied drug distribution by confocal microscopy and the other by quantitative extraction methods of specific cellular structures.[3,4] In general, these studies showed that, for non-drug-resistant cells, DOX localizes to the nuclear compartment, with some vesicular-like distribution within the cytoplasm. Similarly, MTZ, at low concentrations, localizes almost exclusively to the nucleus. Additionally, both DOX and MTZ occasionally display selective localization in both the nuclear and plasma membranes.[1,2] Despite the agreement of the confocal and cell fractionation data, it is reported that DOX undergoes fluorescent quenching upon DNA intercalation. Thus, intracellular fluorescence may not completely reflect the true subcellular distribution of these drugs,

This publication was supported by grant no. GM 49875 from the National Cancer Institute. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Cancer Institute. Confocal microscopy was performed in the Southwest Environment Health Sciences Center microscopy laboratory, supported by USPHS grant ES 06994.

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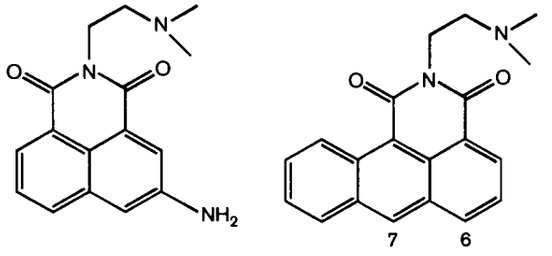
although confocal microscopy has been successfully used to compare drug distribution patterns in sensitive and multidrug-resistant (MDR) tumor cell lines *in vitro*.[5-7]

A new group of antitumor compounds structurally similar to the anthracyclines and MTZ are the azonafides. This series of anthracene-based antitumor agents was derived from prior work by Braña *et al.* on a set of DNA intercalators with a benzisoquinoinedione-type structure.[8,9] The newer azonafide compounds have a structural resemblance to amonafide,[10-13] but possess an anthracene chromophore rather than a naphthalene chromophore. (Figure 1). Substitution around the anthracene nucleus of the parent azonafide compound has produced over 160 analogs to date. These compounds display a wide range of cytotoxic potencies when tested in human and murine tumor cells *in vitro*.[13-15] Prior mechanistic investigations with azonafide showed that antitumor cytotoxic potency is roughly proportional to DNA intercalation potency, as noted by increased stability against thermal denaturation of double-stranded calf thymus DNA.[11-13] Further mechanistic studies showed that these agents produce DNA single-strand breaks, DNA double-strand breaks and DNA-protein cross-links characteristic of topoisomerase II (Topo II) inhibition, as well as catalytic inhibition of Topo II activity.[16] However, the DNA damage produced by these compounds does not correlate with the cytotoxicity of all the analogs studies. This suggests that mechanisms in addition to the DNA effects may be critical for the toxicity of certain azonafide analogs.

The current study was designed to assess the intracellular distribution of several azonafide analogs. Since previous studies of structurally similar DNA intercalators have shown a correlation between decreased cytotoxicity, or the development of drug resistance and altered drug distribution, we felt it conceivable that altered drug distribution might determine cytotoxic potency among different azonafide analogs. In this report, we show that differing substitutions along the anthracene chromophore of the azonafide series of antitumor agents results in distinct intracellular drug distribution patterns.



Amonafide Azonafide

Figure 1. Structures of the DNA-intercalating drugs amonafide and azonafide.

Materials and methods

Drugs

Azonafide analogs were synthesized and purified by thin layer chromatography. The structures were confirmed using NMR and compositional analytical techniques as previously described.[11-13] They were reconstituted into stock solutions of 2 mg/ml (1 mg/ml for analogs 53 and 54) in dimethyl sulfoxide (DMSO) (JT Baker, Phillipsburg, NJ; analytical grade) and kept at −20°C until use. Previous laboratory studies have shown these drugs to be stable when stored in this manner (unpublished data). The analogs chosen for study, and their substitutions, are described in Table 1.

Cell culture

Chinese hamster ovary cells (ATCC CCL 61) were obtained from the ATCC (Rockville, MD). Cells were maintained in PDRG basal media (HyClone, Logan, UT) supplemented with 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin (Irvine Scientific, Santa Ana, CA), and 5% iron-enriched calf serum (Intergen, Purchase, NY). Cells were incubated at 37°C

Table 1. Substitutions of azonafide analogs studied

Analog	Substitution
1	none
35	7-NH_2
37	6-NH_2
46	6-CH_3
47	6-$NHCH_2CH_2N(CH_3)_2$
53	6-OCH_2CH_3
54	6-OCH_3
62	7-$NHCH_2CH_2N(CH_3)_2$
82	6-$NHCOCH_3$
89	6-$NHCH_2CH_2OH$
104	6-$OCH_2CH_2N(CH_3)_2$
132	6-$N(CH_3)_2$
135	6-OCH_2CH_2CH3
136	6-$O(CH_2)_2SCH_3$

in 95% air, 5% CO_2 with 100% relative humidity. Cells were tested bi-monthly and confirmed to be mycoplasma free using a PCR-based mycoplasma detection kit (ATCC).

Scanning laser confocal microscopy (SLCM)

Intracellular drug distribution was studied by examining drug-treated cells under SLCM, using the autofluorescent property of the anthracene nucleus. Briefly, 10^6 cells were seeded onto sterilized glass coverslips and incubated to allow for attachment overnight. The cell-coated coverslips were then exposed to 10 μM of each analog for 1 h at 37°C. After incubation, coverslips were washed 3 times with phosphate-buffered saline (PBS, pH 7.4). Cells were then fixed with 4% neutral buffered formalin for 10 min, followed by two additional washes with PBS at 5 min each. The coverslips were inverted and mounted onto glass microscope slides with fluorescent mounting medium (Dako, Carpinteria, CA). The slides were then observed and imaged using a Leica TCS-4D scanning laser microscope (Heidelberg, Germany) equipped with a $\times$100 oil immersion lens (1.4 numerical aperture). This instrument employs an Ar-Kr laser emitting at a wavelength of 488 nm. The 488 nm laser line was used for excitation in a FITC filter, which allows detection of emitted light at approximately 520 nm. All images (approximately 5 μM horizontal intracellular sections) were obtained with exactly the same scanning parameters, including laser intensity and pinhole sizes. All images were collected at $\times$100 magnification, with the exception of analogs 1, 35, 47, 53 and 104, which were imaged at $\times$200 magnification. Color images were transformed to grayscale images using Adobe Photoshop, version 3.0.5 (Adobe Systems, San Jose, CA.). Each image was presented as a micrograph which was representative of at least five fields imaged for each sample.

Microculture tetrazolium assay

The MTT assay is based on reductive cleavage of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium (MTT) bromide to a colored formazan compound to indicate cell viability.[17] Chinese hamster ovary cells were plated at a density of 1000 cells/well in 96-well microtiter plates (Costar, Cambridge, MA). On day 2, drugs dissolved initially in DMSO were diluted serially with PBS and added to the wells at concentrations of 1 nM to 10 μM, in half-log increments. Final concentrations of DMSO did not exceed 0.1% (v/v). The plates were then incubated at 37°C for 5 days. After the 5 day incubation period, 50 μl of a 1 mg/ml MTT solution was added to each of the wells and the plates were incubated for an additional 4 h at 37°C. The medium was then aspirated and the formazan product was solubilized in DMSO 100 μl/well. The intensity of the purple color formed in viable cells was quantified by absorbance at 540 nm on an automated microculture plate reader (Biomek 1000; Beckman Instruments, Fullerton, CA). Test results were calibrated in percent control absorbance from diluent-treated CHO cells. Each drug concentration was tested in three independent experiments and the IC_{50} values were determined using the dose-response analysis function (sigmoid fit) in a data manipulation software package (Microcal Origin, version 4.10; Microcal Software, Northampton, MA). The same software was also employed for the statistical analysis of the cytotoxicity data using Student's *t*-test and one-way ANOVA.

Results

Intracellular distribution of analogs

All of the analogs in this study (Table 1) were detected within CHO cells using SLCM. The distribution patterns of the analogs varied, but in no case was there an absence of intracellular fluorescence in drug-treated cells. Furthermore, cells that were examined in the absence of drug treatment produced no fluorescence and imaged as completely black micrographs (not shown).

The azonafide analogs distributed within CHO cells in three distinct patterns, perinuclear, nuclear and cytoplasmic. Two analogs comprise the group that displayed perinuclear distribution, the unsubstituted parent compound (analog 1) and the 6-methyl substituted compound (analog 46). While these analogs localized primarily to the nuclear membrane (Figure 2), they also produced fluorescence within the nuclei and cytoplasm of the CHO cells. Eight analogs comprise the predominantly nuclear localizing group of azonafides. With a single exception (analog 104), all of these nuclear localizing analogs are amine-substituted derivatives of azonafide. These include analogs 35, 37, 47, 62, 82, 89 and 132. Analog 104 is an ethoxy-substituted azonafide in which an amine function is found not on the anthracene, but rather

on a alkyl side chains. Except for analog 104, which was localized in the cytoplasm, these ethoxy-substituted analogs produced fluorescence primarily in CHO cell nuclei (Figures 2 and 3). These analogs were also distributed in the cytoplasm of the CHO cells to varying, but lesser extents, than in the nucleus. An excellent contrast involves analog 104 which produced intense fluorescence in the cytoplasm, compared to analog 62 which was almost exclusively localized in the nuclear (Figure 2). The cytoplasmic-localizing group of analogs is made up of four azonafides, all of which are ethoxy substituted

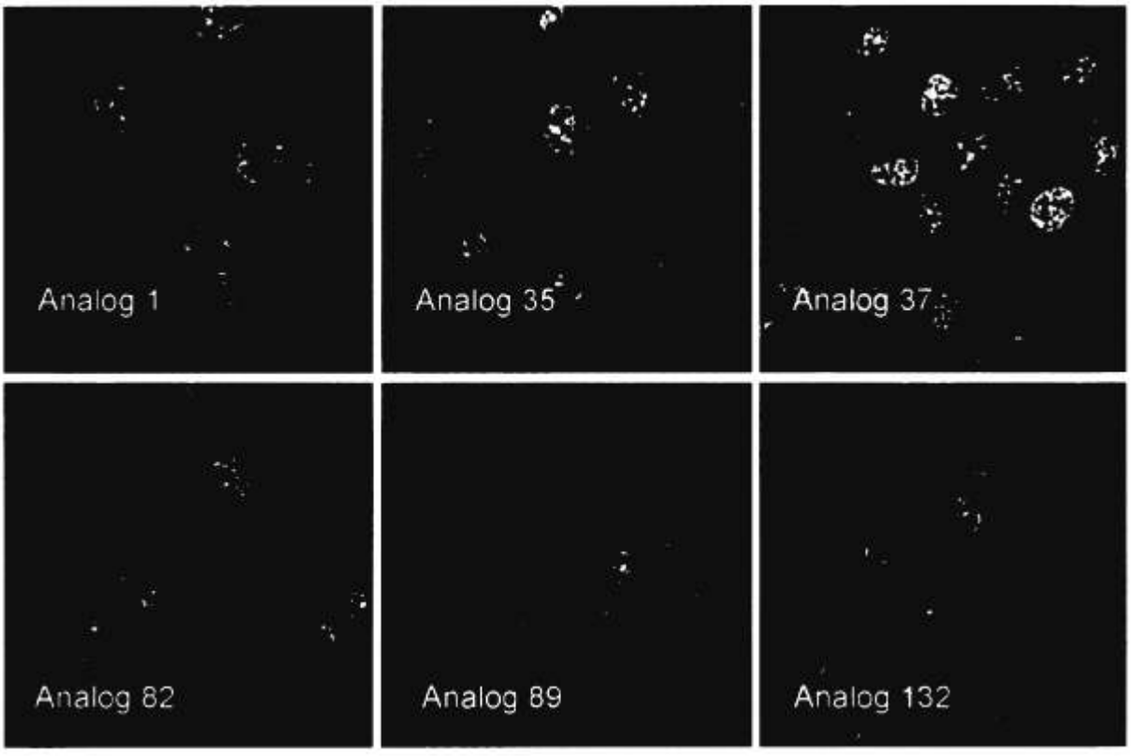


Figure 2. Intracellular fluorescence, detected by SLCM, within CHO cells following a 1 h drug exposure to various perinuclear and nuclear-localizing azonafide analogs (analogs 1, 35, 37, 82, 89 and 132; 10 μM). Images were obtained as described in Materials and methods.

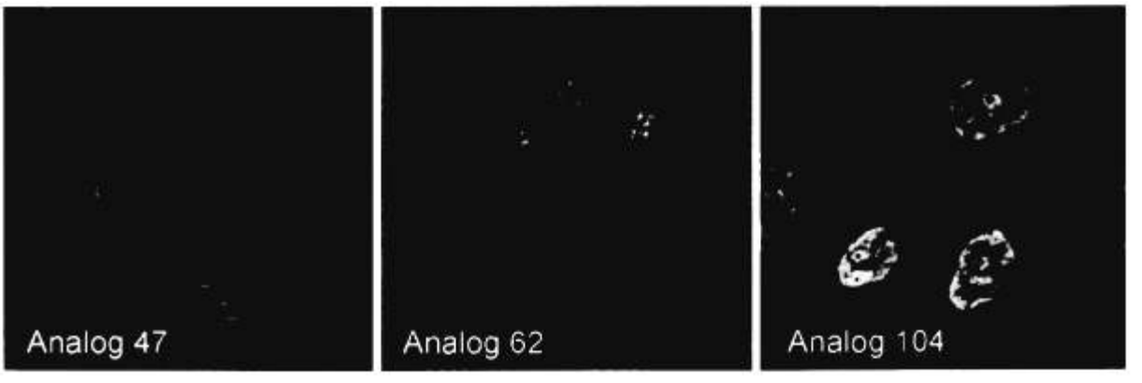


Figure 3. Intracellular fluorescence, detected by SLCM, within CHO cells following a 1 h drug exposure to various nuclear-localizing azonafide analogs (analogs 47, 62 and 104; 10 μM). Images were obtained as described in Materials and methods.

(analogs 53, 54, 135 and 136) (Figure 4). The cytoplasmic distribution of these agents was often not homogenous and several of the drugs displayed a distinctly punctate pattern of cytoplasmic distribution. This includes analogs 50, 53, 135 and 136. Despite their primarily cytoplasmic localization pattern, a small amount of nuclear fluorescence was detected in CHO cells treated with each analog of this group (Figure 4).

Analog cytotoxicity

All of the analogs were analyzed for cytotoxicity towards CHO cells using a 5 day, constant exposure methodology. In these studies the analogs inhibited CHO cell viability with IC_{50} values in the nM range (Table 2). The IC_{50} values ranged from 0.6 ± 3.5 to 224.5 ± 13.0 nM. The rank order of highest to lowest potency among the analogs was $104 = 47 > 35 > 53 > 54 = 1 > 89 = 46 = 37 >$

Table 2. Cytotoxicity of azonafide analogs toward CHO cells in MTT assays[a]

Analog	IC_{50} (nM) [SD]
1	10.7 [0.3]
35	6.4 [0.3]
37	18.2 [1.1]
46	16.8 [2.7]
47	1.2 [2.8]
53	8.1 [0.1]
54	10.6 [0.1]
62	77.1 [1.0]
82	224.5 [13.0]
89	16.5 [0.7]
104	0.6 [3.5]
132	46.7 [1.5]
135	68.0 [1.1]
136	60.1 [0.9]

[a]Continuous drug exposure time was 5 days in the MTT assays. Results are based on three independent experiments for each agent.

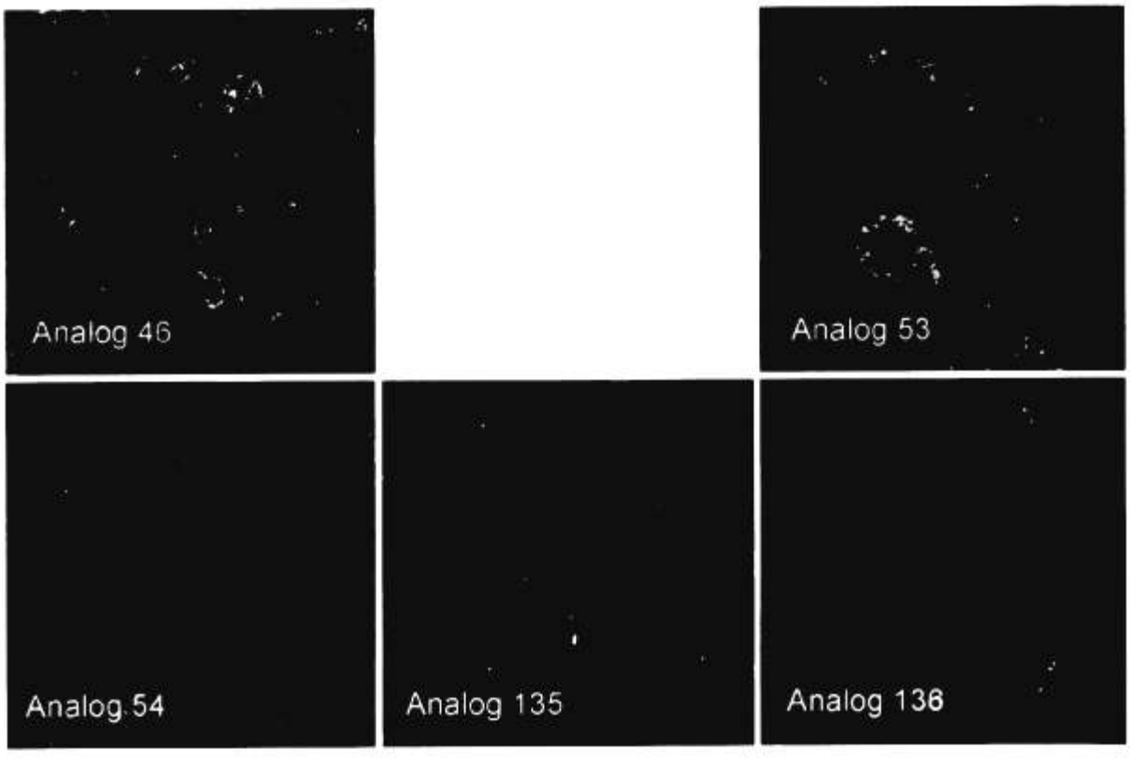


Figure 4. Intracellular fluorescence, detected by SLCM, within CHO cells following a 1 h drug exposure to various perinuclear and cytoplasmic-localizing azonafide analogs (analogs 46, 53, 54, 135 and 136; μM). Images were obtained as described in Materials and methods.

132>136>135>62>>82. In this analysis overlapping means (±SD) were inferred as equal potencies.

Correlation of analog distribution and cytotoxicity

The mean IC_{50} value among analogs within each distribution group was determined. The perinuclear, nuclear and cytoplasmic distribution groups had mean IC_{50} values of 13.8±4.3, 48.9±75.6 and 36.7±31.8 nM, respectively. Individual differences in cytotoxic mean potencies are statistically insignificant as determined using a one-way ANOVA.

Discussion

All of the azonafides tested entered CHO cells very rapidly, and were visualized in both the nuclear and cytoplasmic compartments of CHO cells to varying extents. There were three general patterns of intracellular distribution observed in this study, nuclear, perinuclear and cytoplasmic. Despite the variability in the intracellular distribution patterns between the azonafide analogs, they are all consistent with patterns reported for other DNA intercalators of similar structure. For example, at high concentrations, MTZ localizes within cell nuclei and binds to both DNA and RNA in the nuclear chromatin with a preference for nucleoli.[2,18] In the current study, analogs 35, 37, 47, 62, 82, 89, 104 and 132 localized extensively to the nucleus in a pattern similar to MTZ (Figures 2 and 3). Interestingly, analogs 35, 37, 82 and 89 were not visualized in CHO cell nucleoli in this study, and in this regard differ with MTZ. In contrast, analogs 53, 54, 135 and 136 localized primarily to the cytoplasm with little fluorescence observed in the nuclei. This pattern is similar to that of the anthracyclines DOX and DN, and the anthracene amsacrine. These intercalators have been reported to localize in the cytoplasm in the same vesicular-like distribution pattern seen in the current report,[3,19] as well as to the plasma membrane and nuclear membrane.[1] In contrast, the lesser degree of cytoplasmic fluorescence that was observed for the nuclear-localizing azonafides did not occur in a vesicular pattern (Figures 2 and 3).

The analogs with shared distribution patterns in this study have moieties of similar structure substituted along the anthracene chromophore. For example, the nuclear-localizing analogs are all amine-substituted compounds. The exception is analog 104 which has an ethoxy-substituted (dimethylamino)ethyl side chain. The cytoplasmic-localizing analogs are all ethoxy-substituted analogs. Finally, the two perinuclear-localizing analogs (nos 1 and 46) differ only by the addition of a methyl group at the 6-position for analog 46. The observation that analogs having common substituent structural motifs also shared a common intracellular distribution pattern suggests that there are certain structural requirements for specific subcellular distribution patterns within CHO cells. It appears that substitutions with an amine group results in primarily nuclear-localizing analogs. Substitution of azonafide with an ethoxy substituent results in analogs that either preferentially localize to the cytoplasm or, conversely, are *unable* to enter CHO cell nuclei. It seems more probable that these ethoxy-substituted analogs preferentially localize to the cytoplasm but are not completely excluded from the nucleus.

Finally, some degree of nuclear fluorescence was observed in CHO cells after exposure to all of the analogs in this study, including the cytoplasmic-localizing analogs, and analogs 1 and 46 which had preferential perinuclear localization. The two latter analogs may represent compounds that do not have a cytoplasmic localization preference, like the ethoxy-substituted analogs, but are either unable to efficiently enter the CHO cell nuclei in large amounts or bind avidly to nuclear membrane structures.

The localization of azonafide analogs, particularly those with cytoplasmic and perinuclear localization, within mitochondria cannot be ruled out. Certain strongly basic intercalators such as pyronine Y and ditercalinium are sequestered within mitochondria.[20,21] Additonally, all of the analogs appear to have some localization to the cytoplasmic compartment of the CHO cells and this may reflect mitochondrial accumulation. Accumulation into lysosomes, which has also been reported for anthracycline-based DNA intercalators such as DOX and DN, may also occur with the azonafides.[22] Unfortunately, the SLCM methodology used here is not capable of resolving cellular structures as small as mitochondria or lysosomes.

Studies of DNA intercalators with structures similar to the azonafides have shown that cells with acquired resistance to these agents display an altered intracellular distribution pattern, relative to sensitive cells. For example, Coley *et al.* showed that in both murine mammary tumor cells and human large cell lung cancer cells, drug-sensitive cells had intracellular DOX localized to the nucleus whereas drug-resistant cells had DOX localized mainly in the cytoplasm.[5]. Addi-

tionally, in bladder cancer cell lines, both epirubicin and idarubicin have been reported to localize to cell nuclei in sensitive cells and the cytoplasm in resistant cells.[6,7] Indeed, one form of MDR involving the M_r 110 000 LRP major vault protein is associated with a vesicular/lysosomal pattern of expression and drug retention.[23] A similar lysosomal distribution pattern may occur with the azonafides.

The comparison of cytotoxicity and cellular distribution patterns for this group of azonafide analogs was performed to determine if a particular localization pattern was associated with greater cytotoxic potency. The conclusion from this analysis is that there is no correlation between intracellular drug distribution patterns and cytotoxic potency for the azonafide analogs *in vitro*. For example, analog 104 has over 300-fold greater cytotoxic potency than analog 82, and these two analogs represent the greatest and least potent analogs in this group, respectively. Nonetheless, both analogs localized to CHO cell nuclei with comparable fluorescent intensities. Furthermore, statistical analysis of the mean IC_{50} values of analogs within each distribution group (nuclear, cytoplasmic and perinuclear) failed to detect any significant differences in toxicity based on the localization pattern. In other words, nuclear localizing azonafide analogs are not necessarily the most potent azonafide analogs. This suggests that non-nuclear targets may be involved in mediating cytotoxic effects of this series of agents. The same conclusion was reached in the prior mechanistic study which could not correlate cytotoxic potency with inhibition of Topo II among the five highly active azonafides.[16]

Conclusion

Three types of subcellular drug localization patterns were detected by SCLM for 14 structurally related anthracenes. The amine-substituted compounds localized preferentially to the nucleus. Ethoxy-substituted compounds localized to the cytoplasm and lowly, or unsubstituted compounds, localized in perinuclear pattern. These patterns were not absolute and some drug fluorescence was noted in all three compartments within each group. Cytoplasmic localization was sometimes not homogenous and involved a punctate vesicular distribution patterns. There was no correlation of localization patterns with cytotoxic potency, both within or between the three localization groups.

Based on the findings in the current study, it appears that the localization pattern among the azonafide analogs is greatly influenced by the nature of substitutions along the anthracene chromophore. However, the localization pattern of these drugs within CHO cells does not influence their cytotoxic potency.

References

1. Weaver JL, Pine PS, Aszalos, *et al.* Laser scanning and confocal microscopy of daunorubicin, doxorubicin, and rhodamine 123 in multidrug-resistant cells. *Exp Cell Res* 1991; **196**: 323-9.
2. Smith PJ, Sykes HR, Fox ME, Furlong IJ. Subcellular distribution of the anticancer drug mitoxantrone in human and drug-resistant murine cells analyzed by flow cytometry and confocal microscopy and its relationship to the induction of DNA damage. *Cancer Res* 1992; **52**: 4000-8.
3. Dessypris EN, Brenner DE, Baer MR, Hande KR. Uptake and intracellular distribution of doxorubicin metabolites in B-lymphocytes of chronic lymphocytic leukemia. *Cancer Res* 1988; **48**: 503-6.
4. Roberts RA, Cress AE, Dalton WS. Persistent intracellular binding of mitoxantrone in a human colon carcinoma cell line. *Biochem Pharmacol* 1989; **38**: 4283-90.
5. Coley HM, Amos WB, Twentyman PR, Workman P. Examination by laser confocal fluorescence imaging microscopy of the subcellular localization of anthracyclines in parent and multidrug resistant cell lines. *Br J Cancer* 1993; **67**: 1316-23.
6. Duffy PM, Hayes MC, Gatrell SKE, Cooper A, Smart CJ. Determination and reversal of resistance to epirubicin intravesical chemotherapy: a confocal imaging study. *Br J Urol* 1996; **77**: 824-9.
7. Duffy PM, Hayes MCCA, Smart CJ. Confocal microscopy of idarubicin localization in sensitive and multidrug-resistant bladder cancer cell lines. *Br J Cancer* 1996; **74**: 906-9.
8. Braña MF, Castellano JM, Roldan CM, *et al.* Synthesis and mode(s) of action of a new series of imidine derivatives of 3-nitro-1,8-naphthoic acid. *Cancer Chemother Pharmacol* 1980; **4**: 61-6.
9. Braña MF, Sanz AM, Castellano JM, Roldan CM, Roldan C. Synthesis and cytostatic activity of benz-(*de*)-isoquinoline-1,3-diones. Structure activity relationships. *Eur J Med Chem* 1981; **16**: 207-12.
10. Sami SA, Dorr RT, Alberts DS, Remers WA. 2-Substituted 1,2-dihydro-3H-dibenz[*de,h*]isoquinoline-1,3-diones. A new class of antitumor agent. *J Med Chem* 1993; **36**: 765-770.
11. Sami SA, Dorr RT, Solyom AM, Alberts DS, Remers WA. Amino-substituted 2-[2′(dimethylamino)ethyl[-1,2-dihydro-3H-dibenz[*de,h*]isoquinoline-1,3-diones. Synthesis, antitumor activity, and quantitative structure-activity relationship. *J Med Chem* 1995; **38**: 983-93.
12. Sami SA, Dorr RT, Alberts DS, Solyom AM, Remers WA. 2-[2′(dimethylamino)ethyl[-1,2-dihydro-3H-dibenz[*de,h*]isoquinoline-1,3-diones with substitutions at positions 4, 8, 9, 10, and 11. Synthesis, antitumor activity, and quantitative structure-activity relationships. *J Med Chem* 1996; **39**: 4978-87.

13. Sami SA, Dorr RT, Solyom AM, Alberts DS, Iyengar BS, Remers WA. 6- and 7-substituted 2-[2′(dimethylamino)ethyl[-1,2-dihydro-3H-dibenz[*de,h*]isoquinoline-1,3-diones: synthesis, nucleophilic displacements, antitumor activity, and quantitative structure-activity relationships. *J Med Chem* 1996; **39**: 1609–18.
14. Dorr RT, Alberts DS, Sami SA, Remers WA. Consistent cytotoxic activity in multidrug resistant tumors for azonafide, a new anthracene analog of amonafide. *Proc Am Ass Cancer Res* 1991; **32**: 408 (abstr).
15. Remers WA, Dorr RT, Sami SA, Solyom AM, Alberts DS. Quantitative structure-activity relationships of azonafide analogs. *Proc Am Ass Cancer Res* 1997; **38**: 226 (abstr).
16. Mayr CA, Sami SA, Dorr RT. *In vitro* cytotoxicity and DNA damage production in Chinese hamster ovary cells and topoisomerase II inhibition by 2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3H-dibenz[*de, h*]isoquinoline-1,3-diones with substitutions at the 6 and 7 positions (azonafides). *Anti-Cancer Drugs* 1997; **8**: 245–56.
17. Alley MC, Scudiero DA, Monks A, *et al.* Feasibility of drug screening panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res* 1988; **48**: 589–601.
18. Durr FE, Wallace RE, Citarella RV. Molecular and biochemical pharmacology of mitoxantrone. *Cancer Treat Rev* 1983; **10** (suppl B): 3–11.
19. Baguley BC. DNA intercalating anti-tumor agents. *Anti-Cancer Drug Des* 1991; **6**: 1–35.
20. Darzynkiewicz Z, Kapuscinski J, Carter SP, Schmid FA, Melamed MR. Cytostatic and cytotoxic properties of pyronin Y: relation to mitochondrial localization of the dye and its interaction with RNA. *Cancer Res* 1986; **46**: 5760–6.
21. Segal-Bendirdjian E, Coulaud D, Roques BP, Le Pecq JB. Selective loss of mitochondrial DNA after treatment of cells with ditercalinium (NSC 335153), an antitumor bis-intercalating agent. *Cancer Res* 1988; **48**: 4982–92.
22. Noel G, Peterson C, Trouet A, Tulkens P. Uptake and subcellular localization of daunorubicin and adriamycin in cultured fibroblasts. *Eur J Cancer* 1978; **14**: 363–8.
23. Scheper RJ, Broxterman HJ, Scheffer GL, *et al.* Overexpression of a M_r 110,000 vesicular protein in non-P-glycoprotein-mediated multidrug resistance. *Cancer Res* 1993; **53**: 1475–9.

(Received 20 October 1998; accepted 29 October 1998)