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Biochemical and Pharmacological Activity of Novel 8-Fluoroanthracyclines: Influence of Stereochemistry and Conformation

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SUMMARY

In an attempt to better understand the role of the cyclohexene ring (ring A) in the biochemical and pharmacological properties of anthracyclines related to doxorubicin and daunorubicin, we investigated the effects of introduction of a fluorine atom at position 8 of idarubicin (4-demethoxydaunorubicin) on drug molecular conformation and biochemical and pharmacological activities. The study showed that the stereochemistry of the substituent at position 8 influenced the "half-chair" conformation, so that in the (8*R*)-fluoroepimer the A ring retained the α half-chair conformation, which is the most stable for natural compounds (i.e., daunorubicin and doxorubicin), and the (8*S*)-fluoroepimers preferred the β half-chair conformation. The (8*R*)-fluoroepimer was more effective than the (8*S*)-fluoroepimer and idarubicin in stimulating topoisomerase II-mediated DNA cleavage. Similarly, the epimer with the α conformation was markedly more potent than the (8*S*)-epimer as a cytotoxic agent in a variety of human tumor cell lines and was more effective as an antitumor agent in the treatment of an ovarian carcinoma xenograft. In addition, 8-fluoro derivatives were able to overcome the resistance to doxorubicin in a number of human tumor cell lines expressing different mechanisms of resistance. In conclusion, these findings provide evidence that drug interactions involving the external (nonintercalating) moiety of the anthracycline chromophore play an important role in determining pharmacological properties, including drug ability to induce DNA cleavage, and therefore their antitumor efficacy.

Anthracyclines are the most widely used antitumor agents and show a wide spectrum of activity in human cancers. The most effective agent of this class remains DX, despite the intense effort in analog synthesis (1). Analog development was originally limited by a lack of information on the precise mechanism of antitumor action. In recent years, progress has been made in the elucidation of the molecular basis of the cytotoxic effects of these important antitumor agents (2). Similar to other DNA-intercalating agents, anthracyclines are topoisomerase II inhibitors. The drugs stimulate topoisomerase II-mediated DNA cleavage by inhibiting the DNA religation step of the catalytic reaction and stabilizing an intermediate of the enzyme reaction (the cleavable complex). However, the molecular interactions of the drug in the ternary complex with enzyme and DNA are still unclear, and structural requirements for DX-related anthracyclines for optimal activity remain to be elucidated (3). The recognition that DNA topoisomerase II is the primary target of the cytotoxic action of anthracyclines provides a novel rational basis for the design of effective derivatives. Although drug intercalation may have a role in the mechanism of enzyme inhibition, external interactions involving the sugar residue and the cyclohexene ring (ring A) are expected to critically influence formation of the ternary complex (drug/DNA/topoisomerase II) because the drug could be at the interface between the enzyme active site and the DNA cleavage site (4). In particular, the hydroxyl group at C9 is important for the pharmacological activity of DX-related anthracyclines (5). The hydroxyl group of anthracyclines at C9 also seems to be important for drug interaction with DNA. X-ray diffraction analysis of the drug/DNA complex indicates the presence of a stabilizing interaction, via hydrogen bonding, between the hydroxyl group in C9 of the drug and the N3 of a guanine residue (6). Conceivably, the introduction of a fluorine atom adjacent to the hydroxyl function may affect the strength of the hydrogen bonding, thereby providing analogs with higher affinity for the intercalation site onto double-stranded DNA.

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ABBREVIATIONS: DX, doxorubicin; IDA, idarubicin.

The introduction of a fluorine atom adjacent to that hydroxyl function may affect the strength of the hydrogen bonding and the conformation of ring A. Because the external moieties of the intercalating agent are known to be critical for the ability of the drug to inhibit topoisomerase II, new analogs that are expected to exhibit a different interaction in the ternary complex (drug/DNA/topoisomerase II), via a modified hydrogen binding ability and a conformational preference of ring A, are of major pharmacological interest.

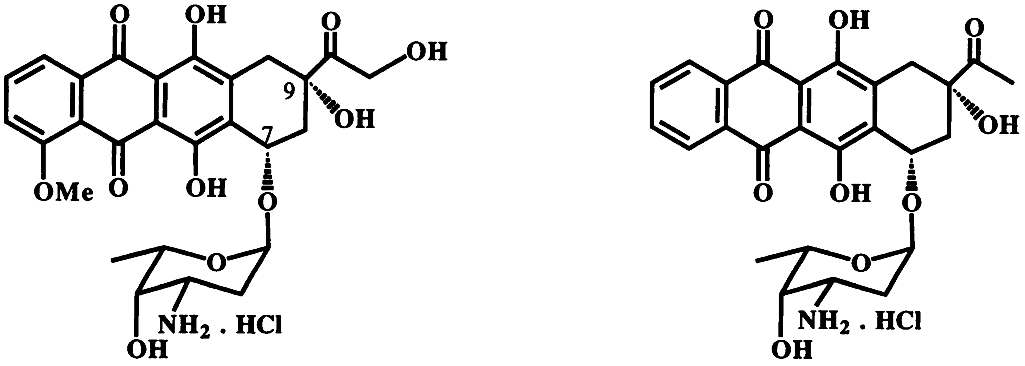
In this study, two epimeric 8-fluoro analogs, (8*S*)-8-fluoroidarubicin and (8*R*)-8-fluoroidarubicin (Fig. 1), were examined to determine the effects of the fluorine on drug conformation and biochemical and biological activities. Cytotoxic activity, antitumor efficacy, and ability to stimulate topoisomerase II-mediated DNA cleavage were compared with those of DX, as the most effective reference compound, and with IDA (4-demethoxydaunorubicin) because the fluoroanthracyclines examined are derivatives of IDA.

Experimental Procedures

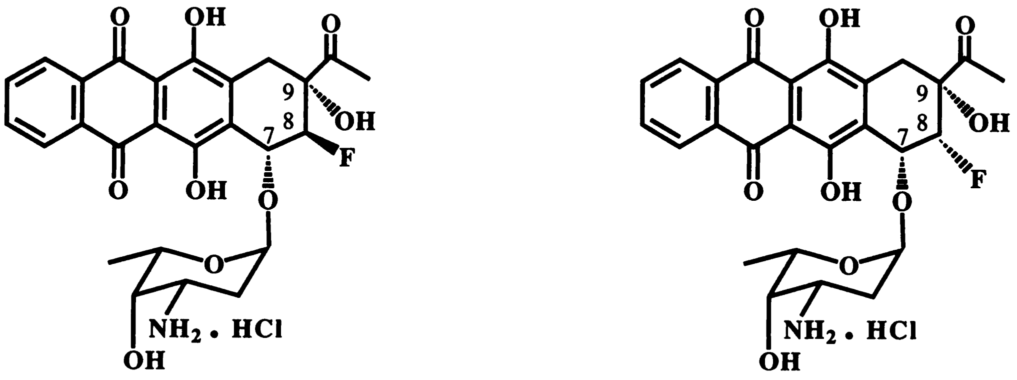
Drugs. The four anthracyclines were synthesized by and obtained from the Department of Chemistry, Menarini Ricerche Sud (Pomezia, Italy). The preparation of the two 8-fluoroanthracyclines requires different synthetic methodologies, which have been reported in detail previously (7). Both compounds were prepared by divergent routes (Fig. 2) starting from the common nephthacenequinone derivative **1** (8). The synthesis of the (8*S*)-fluoroepimer proceeded via epoxidation of the C8—C9 olefinic bond of **1**, oxidation of the resulting epoxyalcohol **2**, oxirane cleavage by a fluorinating agent to give the *trans*-fluorohydroxyketone **3**, followed by the introduction of the hydroxyl moiety at C7, glycosylation of racemic **4** with daunosamine, and separation of the resulting diastereoisomers. Conversely, the synthesis of the (8*R*)-fluoroepimer involved the fluorobromination of the C8—C9 olefinic bond of **1** to give **5** and formation of the C9—C13 epoxide **6**, which, after regioselective hydrolysis and oxidation of the resulting fluorodiol to the corresponding *cis*-fluorohydroxyketone, similarly gave the desired racemic and epimeric fluoroaglycon **7** and, therefore, the corresponding glycoside. The relative orientation of the fluorine atom with respect to the groups at C7 and C9 in the two fluoroanthracyclines was determined by nuclear overhouser effect experiments, whereas the absolute stereochemistry was confirmed on the basis of the similarity of circular dichroism spectra to that of natural daunorubicin.

Drugs were dissolved in 0.9% NaCl immediately before use for *in vitro* assays. For *in vivo* studies, drugs were dissolved in sterile distilled water and administered intravenously in a volume of 10 ml/kg of body weight.

Molecular mechanics and NMR studies. Calculations of molecular mechanics were performed with Discovery Insight software (Biosym, San Diego, CA). The energy of the corresponding conformations was calculated using a modified force field in which an extrapotential was introduced to account for the attractive gauche effect. Theoretical NMR coupling constants were calculated using a modi-



Doxorubicin **Idarubicin**



(8*S*)-8-Fluoroidarubicin **(8*R*)-8-Fluoroidarubicin**

Fig. 1. DX, IDA, and novel 8-fluoro analogs.

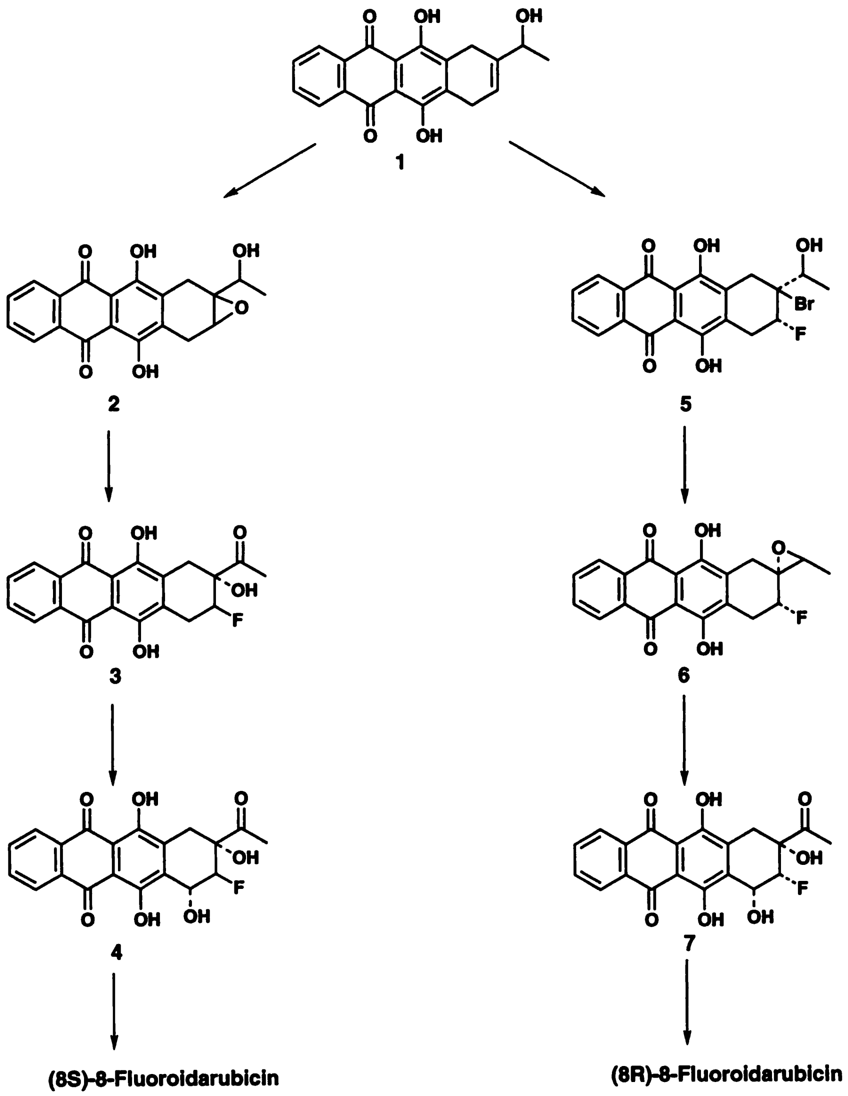


Fig. 2. Synthetic routes to (8*S*)-8-fluoroidarubicin and (8*R*)-8-fluoroidarubicin.

fied Karplus equation (9). Experimental NMR studies were performed on a Varian Gemini 300-MHz spectrometer (Varian, Palo Alto, CA). The spectra were run in $CDCl_3$ and in dimethylsulfoxide-d_6 at concentrations of 2–7 mM. Experimental coupling constants were corrected for possible non-first-order effects according to the LAOCOON routine (10).

Topoisomerase II-mediated DNA cleavage. Simian virus 40 DNA fragments were uniquely 5′-end ^{32}P-labeled as described previously (3). Briefly, simian virus 40 DNA was restricted with the indicated enzyme, dephosphorylated, and ^{32}P-labeled with T4 kinase. Then, DNA was subjected to a second enzyme digestion to generate uniquely 5′-end-labeled fragments, which were separated by agarose gel electrophoresis and purified by electroelution and ethanol precipitation. DNA cleavage reactions were performed in a volume of 20 μl in 0.04 M Tris·HCl, pH 7.5, 0.08 M KCl, 0.01 M $MgCl_2$, 0.005 M dithiothreitol, 1 mM ATP, and 15 mg/ml bovine serum albumin. The DNA fragment was incubated for 30 min with ~32 units of topoisomerase II (200 ng of proteins/2 μl of storage buffer) in the presence or absence of drug. Reactions were stopped by the addition of sodium dodecyl sulfate and proteinase K (1% and 0.25 mg/ml, respectively), and samples were incubated at 42° for an additional 45 min. DNA was then ethanol precipitated, resuspended in 2.5 μl of loading buffer (80% formamide, 10 mM NaOH, 1 mM EDTA, 0.1% xylene cyanol, and 0.1% bromphenol blue), heated at 90° for 2 min, chilled in ice, and then loaded into an 8% polyacrylamide denaturing gel. Gels were run at 80 W for 2 hr, dried, and autoradiographed with Amersham MP-Hyperfilm (Arlington Heights, IL).

Cell lines and cytotoxicity studies. The cell lines used were of human origin and included a squamous cell carcinoma of the cervix (A431), an ovarian carcinoma (A2780), a large cell lung carcinoma (H460), a mammary carcinoma (MCF-7), a colon adenocarcinoma (LoVo), and two small cell lung cancer carcinoma cell lines (POVD and POGB) (3, 11). Cell lines grew adherent to the substrate with the exception of POVD, which grew as floating aggregated cells. The resistant cell lines were obtained after continuous exposure of the parental cell lines to increasing concentrations of DX. All cell lines were cultured in RPMI-1640 (Bio-Whittaker, Verviers, Belgium) containing 10% fetal calf serum (GIBCO BRL/Life Technologies, Gaithersburg, MD).

Cells (5 × 10^4/ml) of all lines except small cell lung cancer were seeded onto six-well plates (Costar, Cambridge, MA), and after 24 hr, samples were treated with the drugs for 1 or 24 hr. At 72 hr after treatment, cells were trypsinized and counted with a cell counter (Coulter Electronics, Luton, UK). The small cell lung cancer cells were treated at cell seeding, and cell survival was determined 96 hr later by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium assay as previously described (3). The results are expressed as mean IC_{50} values of at least three experiments.

***In vivo* studies.** Female athymic Swiss nude mice, 6–10 weeks old, were used in the study. Animals (Charles River Italia, Calco, Italy) were maintained in laminar air flow rooms. Sterilized cages, bedding, and acidified water were used. The air was kept at a temperature of 24–26° with 50% humidity. The experiments were approved by the Institutional Committee for Animal Experimentation. The human ovarian A2780 cells were injected subcutaneously into nude athymic mice. For tumor line maintenance and experimental purposes, tumor fragments were grafted subcutaneously into both flanks of athymic mice with a 13-gauge trocar. Growth of subcutaneous tumors was followed by biweekly caliper measurement of tumor length and width. Tumor weight was calculated in milligrams using the formula: tumor weight = $width^2 \times length/2$ (12). Chemotherapy studies were carried out on mice bearing subcutaneously tumors transplanted in both flanks, and each experimental group consisted of at least eight assessable tumors. Treatments were delivered intravenously every 7 days for a total of three treatments. Drug activity was evaluated as percentage of tumor weight inhibition at 7–8 days after the last drug treatment [calculated as $100 - (T/C \times 100)$, where T is the mean tumor weight of treated tumors and C is the mean tumor weight of control tumors], and log cell kill [calculated by assessing the difference in mean time required for tumors of treated and control mice to reach 2000 mg and dividing that value by the product of 3.32 and mean doubling time of the control tumors (6 and 8 days in the two experiments)]. Student's *t* test (two tailed) was used for statistical comparison of tumor weight in mice treated with 8-fluoroanthracycline versus DX-treated mice.

Results

Conformational analysis. The results of previous studies, dealing with the conformation of antitumor anthracyclines in the solid state, gave indications that the cyclohexene ring (ring A) was in the half-chair conformation, as also shown by the ^{1}H NMR spectra of different daunorubicin derivatives, with the sugar moiety nearly perpendicular to the plane of the chromophore (13). The conformation of ring A can be important for the interaction of these drugs with DNA, and it is believed to be a relevant structural property for antitumor activity (14). Existence of the preferred α half-chair conformation of ring A in daunorubicin (Fig. 3) and DX in solution has been proved with ^{1}H NMR (15, 16). Two interactions were thought to favor the α half-chair conformation: the possibility of hydrogen bonding between 9-OH and 7-O, and the longer distance between phenolic 6-OH and 7-O, which decreases the repulsive interaction between the two corresponding dipoles. In this respect, semisynthetic 6-deoxyanalogues, which were shown to assume preferentially the β half-chair conformation for ring A by ^{1}H NMR studies in polar solvents, have a lower affinity for native DNA than daunorubicin (14). Molecular mechanics calculations performed to determine the relevant conformational forms of ring A of IDA, of the (8*S*)-fluoro analog, and of its corresponding (8*R*)-fluoroepimer indicate a preference for the α half-chair conformation for IDA and a preference for the β half-chair conformation for the (8*S*)-fluoro analog. This finding is noteworthy because the only example in the literature of a preferred β half-chair conformation for ring A of anthracyclines is that of the 6-deoxy derivatives in dimethylsulfoxide (14). Calculations also predict a propensity for the α half-chair conformation for the (8*R*)-fluoroepimer, suggesting that inversion of the configuration at the carbon atom bearing the fluorine substituent would make the (8*R*)-fluoroanthracycline assume the conformational preference of the known antitumor anthracyclines. The calculated vicinal NMR coupling constants, $^3JH7\text{-}H8$, for the two conformers (α half-chair and β half-chair, respectively) of IDA and of the two 8-fluoroepimers were in keeping with the experimental data obtained for the same vicinal coupling constants for the three compounds in deuterated dimethylsulfoxide solutions. In the case of IDA, the population of the α half-chair conformers was 100%, whereas in the case of the two 8-fluoroepimers, we obtained 66% of β conformation for the (8*S*)-8-fluoro analog and 100% of α conformation for the (8*R*)-8-fluoro analog. In the (8*S*)-fluoroanthracycline, the importance of the β half-chair conformation in equilibrium could be due to a stereoelectronic attractive gauche effect between 7-O—8-F and 9-OH—8-F. Conversely, the same effect would command a propensity of the α half-chair conformation for the (8*R*)-fluoroepimer (Fig. 3).

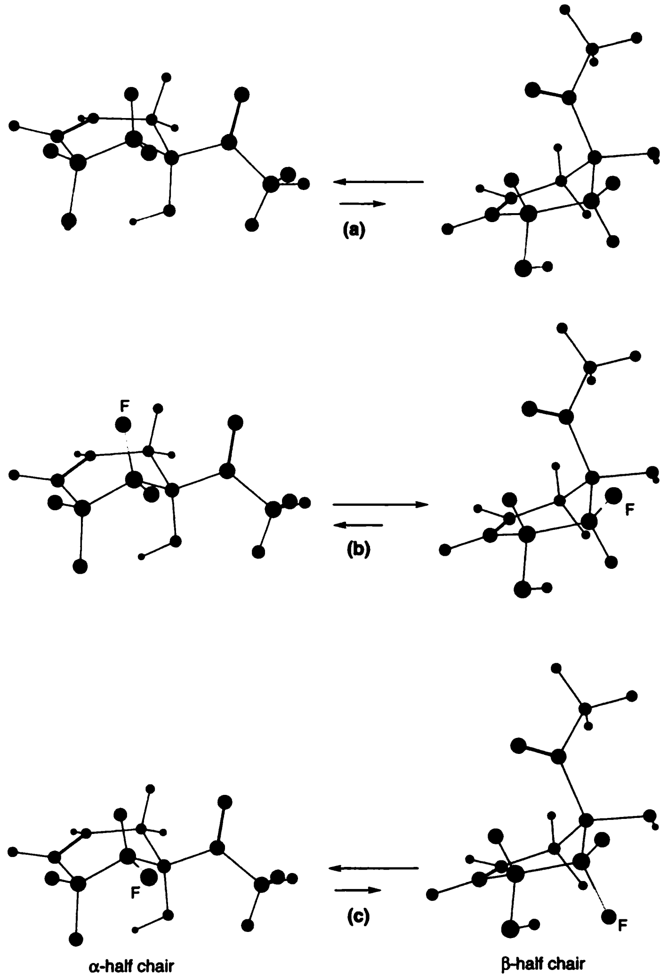


Fig. 3. Conformational flexibility of ring A of anthracyclines (a), (8*S*)-8-fluoroidarubicin (b), and (8*R*)-8-fluoroidarubicin (c).

Stimulation of topoisomerase II DNA cleavage. Drug stimulation of DNA cleavage was investigated by incubating 5′ ^{32}P-labeled simian virus 40 DNA fragments with topoisomerase II and different concentrations of the fluoro derivatives and by analyzing DNA cleavage intensity patterns with polyacrylamide denaturing gels (Fig. 4). Drug effects on topoisomerase-mediated DNA cleavage were compared with those of optimal concentrations of IDA, which has been shown to stimulate DNA cleavage intensity patterns identical to DX. The fluoroderivatives stimulated DNA cleavage at the same sites as IDA with a biphasic dose-response curve.

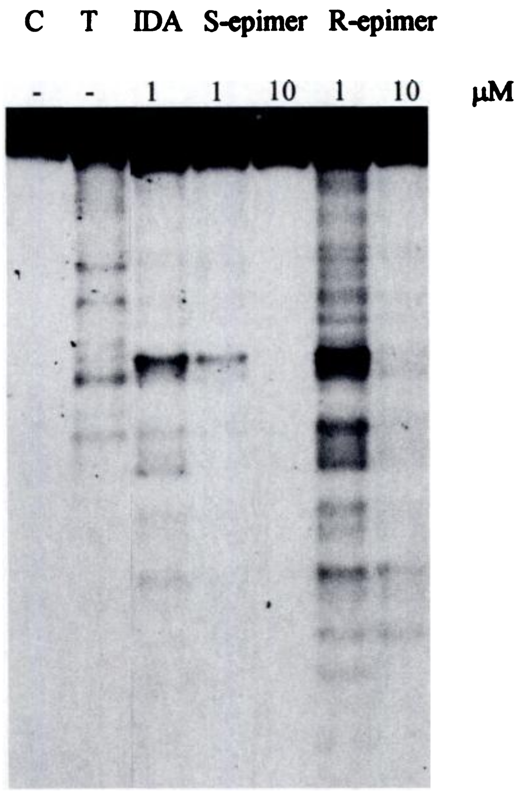


Fig. 4. Topoisomerase II-mediated DNA cleavage stimulated by anthracycline derivatives. A simian virus 40 DNA fragment ^{32}P labeled at one 5′-end was reacted with murine topoisomerase II at 37° for 20 min with or without IDA, *S*-epimer, and *R*-epimer at the concentrations indicated. The reaction was then stopped with sodium dodecyl sulfate and proteinase K (0.1% and 100 μg/ml, respectively). *C*, Control DNA; *T*, DNA reacted with enzyme alone.

Indeed, as already reported for other intercalating anthracyclines (2), strong DNA binding caused inhibition of enzyme catalytic activity at high drug concentrations (i.e., 10 μM). The most interesting finding of the study was a markedly different efficacy of the two epimers in stimulating DNA cleavage. In fact, although the introduction of a fluorine atom in the *S* configuration only slightly reduced drug stimulation activity for DNA cleavage, the (8*R*)-fluoroepimer was more effective than IDA at the same drug level.

Cytotoxic activity and cross-resistant pattern. A comparative evaluation of the cytotoxic activity of the two 8-fluoroanthracyclines was performed in a panel of cell lines of different tumor types, including A431 (cervical carcinoma), H460 (lung carcinoma, non-small cell lung cancer), and A2780 (ovarian carcinoma). The IC_{50} values after 1 or 24 hr of exposure are summarized in Table 1 and compared with the IC_{50} values of reference compound DX or IDA. H460 was the most sensitive cell line to the four drugs after 1 hr of exposure. As expected, IDA was markedly more cytotoxic than DX. Cytotoxicity of the (8*S*)-fluoroepimer was comparable to that of DX, whereas cytotoxic potency of the (8*R*)-fluoroepimer was similar to that of IDA. The IC_{50} values of the drugs were generally lower after 24 hr than after 1 hr of treatment, except for IDA. Table 2 summarizes the pattern of cross-resistance for the four tested anthracyclines in a variety of DX-resistant cell lines expressing different resistance factors and characterized by a different resistance index. As expected for the lipophilic anthracyclines, IDA was able to partially overcome resistance in most of resistant cell systems. A reduced cross-resistance was also shown to the (8*S*)-fluoroepimer, which exhibited a somewhat comparable ability to overcome resistance. In contrast, the (8*R*)-fluoroepimer was found to be able to almost completely overcome resistance in all the cell systems examined. This behavior was most evident in small cell lung cancer cell lines (Table 2), which overexpress *MDR1* (POVD/DX) and *MRP* genes (POGB/DX) (3, 11). Furthermore, the ability of the (8*R*)-fluoroepimer to overcome DX resistance seemed to be dependent on the degree of resistance, as documented in MCF-7/DX and LoVo/DX systems characterized by multidrug-resistant phenotype with a very high resistance index. Multiple resistance factors are likely involved in these cell lines.

Antitumor activity. Table 3 summarizes the antitumor data of the anthracyclines against the A2780 human ovarian carcinoma xenograft. Two independent experiments were performed to evaluate the antitumor activity of each fluoroepimer in comparison to DX. In experiment A, the treatments were started in mice bearing established tumors (mean tumor weight, 350 mg). DX at its optimal dose (7 mg/kg intravenously for three treatments) inhibited tumor growth by 70% and achieved 1 log cell kill. The (8*S*)-fluoroepimer was better tolerated than DX and had the same effect on tumor growth. In experiment B, the activity of the (8*R*)-fluoroepimer was compared with that of DX and IDA; treatments were started in mice bearing small tumors (mean tumor weight, 100 mg). For this reason, DX was more active than in experiment A, and the fluoro derivative was as effective as DX on percentage of tumor weight inhibition (93% versus 83%, $p > 0.05$) and more effective in log cell kill (3 versus 1.8). In addition, the (8*R*)-fluoroepimer was much more potent than DX, with maximal tolerated doses of 1.5 versus 7 mg/kg. IDA was inactive against this human tumor xenograft. No mice died as a result of toxicity in the two experiments, and

TABLE 1

Cytotoxic activity of 8-fluoroanthracyclines on human tumor cell lines

Cell line	Exposure time	IC_{50}[a]			
		Doxorubicin	Idarubicin	(8*S*)-Fluoro epimer	(8*R*)-Fluoro epimer
	hr	*ng/ml*			
A431	1	260	10	220	30
H460	1	60	1	80	25
A2780	1	330 ± 110	5.8 ± 3	200	3.5 ± 1.5
A431	24	30 ± 1	5.3 ± 0.4	35 ± 35	3.5 ± 0.7
H460	24	20 ± 14	3 ± 0.4	22 ± 20	1.8 ± 1
A2780	24	13 ± 4	2.8 ± 2	14 ± 8	5 ± 4

[a] Mean of at least three experiments when ± standard deviation is indicated.

TABLE 2

Pattern of cross-resistance to 8-fluoroanthracyclines of doxorubicin-resistant human tumor cell lines

Cell line	Exposure time	IC_{50}[a]							
		Doxorubicin	RI[b]	Idarubicin	RI	(8*S*)-Fluoro epimer	RI	(8*R*)-Fluoro epimer	RI
	hr	*ng/ml*							
A2780	24	13 ± 4		2.8 ± 2		14 ± 8		5 ± 4	
A2780/DX	24	250 ± 1	19	10 ± 1	3	60 ± 14	4	9 ± 6	2
LOVO	24	41 ± 5		3 ± 1		12 ± 5		3 ± 2	
LOVO/DX	24	989 ± 62	24	20 ± 8	7	147 ± 69	12	11 ± 4	4
MCF-7	24	13 ± 3		2 ± 1		6 ± 2		3 ± 2	
MCF-7/DX	24	2460 ± 610	189	27 ± 16	13	291 ± 81	48	16 ± 12	5
POVD	96	37 ± 7		4 ± 0.1		6 ± 2		9 ± 2	
POVD/DX	96	350 ± 90	9	48 ± 10	12	70 ± 40	12	9 ± 4	1
POGB	96	16 ± 10		2 ± 0.4		20 ± 12		2 ± 1	
POGB/DX	96	390 ± 80	24	24 ± 3	14	140 ± 7	7	4 ± 1	2

[a] Mean ± standard deviation of at least three experiments.

[b] RI, resistance index.

TABLE 3

Antitumor activity of 8-fluoroanthracyclines on the A2780 human ovarian carcinoma xenograft

Compound	Dose[a]	Tumor weight inhibition[b]		Log cell kill[c]	
		Exp. A[d]	Exp. B[d]	Exp. A[d]	Exp. B[d]
	mg/kg	%			
Doxorubicin	7	71	83	1	1.8
Idarubicin	1.2		36		0.3
(8*S*)-Fluoro epimer	8	72		0.8	
	10.4	73		0.9	
(8*R*)-Fluoro epimer	1		72		1.2
	1.5		93		3

[a] Drugs were delivered intravenously every 7 days for a total of three treatments.

[b] Percentage of tumor weight inhibition in treated versus control mice. See Experimental Procedures for calculation.

[c] Log cell kill as a result of the treatment. See Experimental Procedures for calculation.

[d] Exp. A refers to tumors weighing ≥350 mg when drug treatments began; Exp. B refers to tumors just measurable (<100 mg) at the beginning of treatment.

a body weight loss of 4% was observed in both groups of animals treated with the two fluoro derivatives.

Discussion

The results of the current study indicated that the introduction of a fluorine atom at position 8 of the chromophore and its stereochemistry conferred peculiar features to the anthracyclines. Indeed, in both examined 8-fluoroepimers, this modification resulted in significant efficacy in the treatment of a solid tumor system (i.e., human ovarian carcinoma xenograft), comparable or superior to that of DX. This pharmacological behavior is unexpected for a daunorubicin derivative because a general finding in the pharmacology of anthracyclines is a very low activity of daunorubicin analogs against solid tumors (1, 13). The different antitumor profiles of daunorubicin and DX are still unexplained. Furthermore, the presence of a fluorine atom at C8 was associated with a reduction in or lack of cross resistance in DX-resistant cell systems. This modification in the pharmacological profile of the anthracycline derivatives (i.e., efficacy against a solid tumor and ability to partially overcome resistance) was more evident for the (8*R*)-fluoroepimer. In fact, despite lack of the hydroxyl group at position 14, the analog exhibited an antitumor efficacy superior to that of DX. Cytotoxicity results on some human tumor cell lines indicated that the (8*S*)-fluoro derivative was characterized by a decreased potency in comparison to the parent molecule IDA, whereas the (8*R*)-fluoro derivative was not. Furthermore, the IC_{50} values obtained after the 24-hr treatment were lower than those obtained after the 1-hr treatment for the two derivatives and for DX, suggesting that such modifications do not affect the cellular pharmacokinetic properties compared with DX. This point is under investigation.

The different pharmacological properties of 8-fluoroepimers were not related to a change of DNA binding affinity because comparable binding parameters were found for the two epimers (not shown). In contrast, the improved biological behavior of the (8*R*)-fluoroepimer is consistent with the increased ability to stimulate DNA cleavage. If this interpretation is correct, the main conclusion of our study is that a relevant structural feature for optimal biological and biochemical activity of anthracyclines is the conformation of the cyclohexene ring (ring A). Indeed, in the (8*R*)-fluoroaglycon, ring A retains the α half-chair conformation, which has been found in the natural compounds (DX and daunorubicin). In contrast, the (8*S*)-fluoroepimer prefers the β half-chair conformation. Thus, the conformational preference of ring A resulted in a markedly different efficacy in stimulation of DNA cleavage as a consequence of topoisomerase inhibition. Recently, we reported that substituents at the 3′ position of the sugar critically influence the ability of the drug to trap topoisomerase II (3). Thus, the current study provides further evidence that external interactions involving the sugar residue and the cyclohexene ring play a critical role in determining drug ability to induce topoisomerase II-mediated DNA cleavage (17). Relevant to this point is the observation that both ring A and sugar residue are located in the minor groove (18).

The ability of fluoro derivatives to overcome resistance in cell lines with multidrug-resistant phenotype makes them very interesting compounds. Reasons for this behavior, which is more marked for the (8*R*)-epimer, are still unknown and will be investigated. Speculations can be made for *MRP*-expressing cells. The need of a metabolic activation, through C7, has been suggested for DX to allow conjugation with glutathione and transport by the ATP-dependent *MRP*/glutathione pump (19). The presence of a fluorine atom in C8 might interfere with this process and maintain higher intracellular drug level.

In conclusion, our study is consistent with a model of drug interaction in the ternary complex in which the intercalating agent, placed at the interface between the topoisomerase II active site and the DNA cleavage site, exposes its external moieties to the contact with the enzyme and provides a rational basis for the development of new effective anthracyclines.

Acknowledgments

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