Doxorubicin Disaccharide Analogue: Apoptosis-Related Improvement of Efficacy In Vivo

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**Background:** Although doxorubicin remains one of the most effective agents for the treatment of solid tumors, there is an intensive effort to synthesize doxorubicin analogues (compounds with similar chemical structures) that may have improved antitumor properties. We have synthesized a novel doxorubicin disaccharide analogue (MEN 10755) and have characterized some of its relevant biochemical, biologic, and pharmacologic properties.

**Methods:** The antitumor activity of this compound (MEN 10755) was studied in a panel of human tumor xenografts, including xenografts of A2780 ovarian tumor cells, MX-1 breast carcinoma cells, and POVD small-cell lung cancer cells. MEN 10755 was compared with doxorubicin according to the optimal dose and schedule for each drug. The drug’s cytotoxic effects, induction of DNA damage, and intracellular accumulation were studied in A2780 cells. DNA cleavage mediated by the enzyme topoisomerase II was investigated in vitro by incubating fragments of simian virus 40 DNA with the purified enzyme at various drug concentrations and analyzing the DNA cleavage-intensity patterns. Drug-induced apoptosis (programmed cell death) in tumors was determined with the use of MX-1 and POVD tumor-bearing athymic Swiss nude mice.

**Results:** MEN 10755 was more effective than doxorubicin as a topoisomerase II poison and stimulated DNA fragmentation at lower intracellular concentrations. In addition, MEN 10755 exhibited striking antitumor activity in the treatment of human tumor xenografts, including those of the doxorubicin-resistant breast carcinoma cell line MX-1.

**Conclusions:** The high antitumor activity of MEN 10755 in human tumor xenografts, including doxorubicin-resistant xenografts, and its unique pharmacologic and biologic properties make this disaccharide analogue a promising candidate for clinical evaluation. [J Natl Cancer Inst 1997;89:1217–23]

Doxorubicin still remains one of the most effective antitumor agents in clinical use and has the widest spectrum of antitumor activity (1). The observation concerning the minor molecular change differentiating doxorubicin from daunorubicin (the first discovered anthracycline clinically useful against malignant hematologic diseases) and the clinical success of doxorubicin in the treatment of solid tumors have stimulated an intensive effort in analogue synthesis with the aim of improving antitumor properties. Despite the formidable effort, expressed as time and expense devoted to the systematic modification of the parent compound(s), “a truly better doxorubicin has not been found” (2). Unfortunately, in cancer drug discovery and development, analogue synthesis often does not yield anything better than the original lead compounds.

In early attempts to identify anthracyclines more effective than doxorubicin, both empiric and rational approaches have been combined. Although chemical
modifications were based largely on structure–activity relationship studies (1), synthetic efforts were hampered by the late identification of the primary cellular target of drug action, i.e., DNA topoisomerase II, and by the lack of characterization of the ternary complex, drug–DNA–enzyme, which could provide a rational basis for the design of compounds endowed with optimal drug–target interaction (3). Early studies on structure–activity relationships of anthracyclines (1,4) have shown an important role for the structure and stereochemistry of the amino sugar in the biochemical and pharmacologic activities of anthracyclines related to doxorubicin. The presence of the amino group at C-3' has been implicated as a determinant of DNA binding affinity (3). The results from some studies (5–7), however, indicated that the presence of the amino group is not a strict requirement for the pharmacologic activity of anthracyclines. Although substitution of the amino group for a hydroxyl group appreciably reduces drug affinity for DNA, lack of the amino group increases the drug’s ability to stimulate DNA cleavage mediated by topoisomerase II (7). Indeed, the non-intercalating moieties of doxorubicin are expected to critically influence the formation of the ternary complex, since in the latter the drug has been proposed to be positioned at the interface of the active site of the enzyme and the DNA cleavage site (3).

In this report, we describe the synthesis, molecular conformation, and some relevant biochemical, biologic, and pharmacologic properties of the selected analogue of a new series of anthracyclines in which the amino sugar moiety appears as the second residue bound in axial orientation to the first residue, which in turn is characterized by the substitution of the amino group for a hydroxyl group. The disaccharide structural variant was designed with the aim of expanding the recognition options in this portion of the molecule. Bioactivity as a consequence of this type of structural modification in the idarubicin-related series was highly dependent on the orientation of the second, aminated, sugar moiety, whether axial or equatorial (Arcamone F, Animati F, Bigioni M, Capranico G, Caserini C, De Cesare M, et al.: manuscript in preparation).

Since the presence of an additional sugar in the disaccharide analogue is expected to reduce cellular uptake, the demethoxy derivatives of this series appeared to have a more favorable behavior than the derivatives with natural aglycone. The selected compound 4-demethoxy-7-O-[2,6-dideoxy-4-O-(2,3,6-trideoxy-3-amino-α-L-lyxo-hexopyranosyl)-α-L-lyxo-hexopyranosyl]-adriamycinone, i.e., MEN 10755, the first example of a doxorubicin disaccharide analogue, was characterized by its marked ability to induce DNA breaks in tumor cells (apparently as a consequence of topoisomerase II poisoning) and in our experience exhibited unprecedented superior activity compared with doxorubicin in a number of human tumor xenografts growing subcutaneously in athymic mice.

Materials and Methods

Chemical Synthesis

The novel doxorubicin disaccharide analogue was synthesized as indicated in Fig. 1 upon coupling of the aglycone with the disaccharide moiety. The latter, p-methoxybenzyl 3-O-allyloxycarbonyl-4-O-(3-N-allyloxycarbonyl-4-O-p-nitrobenzoyl-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)-2-deoxy-α-L-lyxo-hexopyranoside (4a in Fig. 1), α anomer [α]D —216.1 °, was obtained in almost 90% yield by the reaction of (8) of p-methoxybenzyl 3-O-allyloxycarbonyl-2-deoxy-α-L-fucose (1 in Fig. 1) (9) with thiopephn 3-N-allyloxycarbonyl-4-O-p-nitrobenzoyl α and β-L-daunosaminides (2 in Fig. 1), α anomer [α]D —221.6 °, H1 nuclear magnetic resonance (NMR) (DMSO-d6) d 7.4 (m, 5H, C6H5), 5.92 (d, 1H, H-1), 1H, 16ax = 5.3 Hz), which was in turn prepared in 83% yield after chromatography by treatment of the N-allyloxycarbonyldaunosamin-1,4-di-O-p-nitrobenzoyl derivative (3 in Fig. 1) with phenylthiohydantoin in the presence of trimethylsilyl triflate. Selective removal of the p-methoxybenzyl group in 4a (Fig. 1) followed by p-nitrobenzylation afforded quantitatively 4b (Fig. 1), α anomer [α]D —208.9 °. The final glycosylation reaction on 14-acetoxyidarubicone (5 in Fig. 1) (10) (yield 50%) was followed by stepwise removal of the protecting groups and chromatographic purification to give MEN 10755 (6 in Fig. 1), isolated as the hydrochloride, mp 163–165 ° (differential scanning calorimetry), in 77% yield. All compounds described gave elemental analysis, mass, and NMR spectra in agreement with the assigned structures. Optical rotations were measured at 20 °C (c 0.01, CHCl3). NMR spectra were recorded by use of a 300-MHz spectrometer. Mass spectra were recorded on a VG Quattro apparatus (Micromass, Altrincham, U.K.).

NMR Spectra

The proton magnetic resonances of MEN 10755 were assigned by standard correlated spectroscopy, total correlation spectroscopy, and nuclear overhauser and exchange spectroscopy (NOESY) experiments on an AMX 500-MHz spectrometer (Bruker, Karlsruhe, Germany) by use of a 7 mM solution of the compound in dimethyl sulfoxide-d6 at 25 °C. NOESY spectra were recorded at four mixing times (70, 100, 150, and 200 minutes) to check the linearity of the cross-relaxation buildup. Interproton distances were calculated by use of the initial rate approximation according to a two-spin model. The JCH coupling constants through the glycosidic bonds were measured by the application of pulse sequence a, as proposed by Poppe and van Halbeek (11). Negative nuclear overhauser enhancement (NOE) regime in the NOESY spectra (typical of macromolecular species) and changes in chemical shifts on increasing the temperature suggested the presence of an association. Experimental interproton distances in A (‘‘virtual distances’’ determined by the averaging of the NOEs produced by different conformers in rapid equilibrium) of MEN 10755 and associated errors are as follows: H-1, H-7 = 2.23 (0.11); H-5, H-8an = 2.54 (0.25); H-1, H-4 = 2.60 (0.25); H-1, H-6 = 2.90 (0.30); H-3, H-5 = 2.50 (calibration distance). All other cross-peaks agreed with the conformations assigned via the coupling constants and served as a control for the reliability of the data collected. The three-bond heteronuclear coupling constants (in hertz) measured were (error 0.5 Hz) as follows: C-1, H-7 = 5.7, C-7, H1' = 4.9, C-1', H-4' = 5.9, C-4', H-1' = 3.3.

Molecular Mechanics

A systematic search for ϕ and ψ (in steps of 12°) for a simplified analogue of MEN 10755, in which the daunosamine moiety is replaced by a methyl group, was performed. Eight energy minima were obtained. Each of them was employed as a starting point to perform for MEN 10755 a systematic search for ϕ and ψ in steps of 18°. The conformers obtained were minimized and then clustered into 54 families. The AMBER* force field (12) of DISCOVER (Biosym, San Diego, CA) was used for the calculations. Atomic partial charges were obtained from a fitting of the electrostatic potential calculated at the semiempirical PM3 level. All nonbonded interactions were calculated without any cutoff by use of a distance-dependent dielectric function € (r) = 4.0 € r and a scale factor of 0.5 for the 1-4 van der Waals and electrostatic interactions.

Cytotoxicity Studies

The human A2780 ovarian tumor cell line was maintained in RPMI-1640 medium plus 10% fetal calf serum. Twenty-four hours after seeding, cells were exposed to the drug for 1 hour. At 72 hours after drug treatment, cells were trypsinized and counted with a cell counter (Coulter Electronics, Luton, U.K.). IC50, (i.e., the drug concentration required to inhibit cell growth by 50%) was determined from the dose–response curve.

Intracellular Drug Accumulation

A2780 cells treated for 1 hour with drugs at the indicated concentrations were washed with cold saline buffer and lysed with distilled water and AgNO3 (3% final concentration). Drugs were extracted with water-saturated normal butyl alcohol, and the fluorescence intensity of extracts was measured by means of a fluorescence spectrophotometer (The Perkin-Elmer Corp., Norwalk, CT) at the following excitation and emission wavelengths: 475/585 nm.
for doxorubicin and 478/566 nm for MEN 10755. Intracellular drug concentration was expressed as picomoles per 10^6 cells quantified with respect to a calibration curve.

**Alkaline Elution**

DNA single-strand breaks induced by drugs in A2780 cells were studied by the alkaline elution technique, essentially as reported by Kohn (13). Briefly, exponentially growing cells were labeled with 0.05 μCi/mL [14C]thymidine for 24 hours at 37 °C and chased in nonradioactive medium for 24 hours before drug exposure. Labeled cells were exposed to the indicated concentrations of anthracyclines for 1 hour, washed with Ca^2+-, Mg^2+-free phosphate-buffered saline, and then detached. Cells were gently loaded on a 2-μm-pore-size polycarbonate membrane filter (Millipore Corp., Bedford, MA) and lysed with 0.025 M EDTA (pH 9.7), 2% sodium dodecyl sulfate (SDS), and 0.5 mg/mL proteinase K. The elution buffer consisted of 0.02 M EDTA and 0.1% SDS, adjusted to pH 12.1 with tetrapropylammonium hydroxide. DNA single-strand breaks were expressed as rad-equivalents by use of standard calibration curves obtained with cells exposed to γ-rays.

**Analysis of p53 Gene**

The status of the p53 gene (also known as TP53) of human tumor models was assessed by DNA single-strand conformation polymorphism (SSCP) analysis for the presence of mutations in the most frequently affected exons (exons 5–9) of the gene, as described previously (14).

**Topoisomerase II-Mediated DNA Cleavage**

Immediately before use, drugs were diluted in deionized water. DNA topoisomerase II was purified from the nuclei of P388 murine leukemia cells by published procedures (7) and was stored at −20 °C in storage buffer (20 mM KH_2PO_4 [pH 7.0], 50% glycerol, 0.5 mM phenylmethylsulfonyl fluoride, 0.1 mM EDTA, and 1 mM β-mercaptoethanol). Simian virus 40 (SV40) DNA fragments were uniquely 5'-end-labeled as previously described (7), separated by agarose gel electrophoresis, and purified by electroelution. DNA cleavage reactions were performed in 20 μL of 40 mM Tris–HCl (pH 7.5), 80 mM KCl, 10 mM MgCl_2, 0.5 mM dithiothreitol, 1 mM adenosine triphosphate, and 15 μg/mL bovine serum albumin, with topoisomerase II and anthracyclines at 37 °C for 20 minutes. Reactions were stopped with 1% SDS and 0.1 mg/mL proteinase K, and the mixture was further incubated at 42 °C for 45 minutes. DNAs were then precipitated in ethanol, resuspended in 2.5 μL of 80% formamide, 10 mM NaOH, 1 mM EDTA, and 0.1% dyes, heated at 95 °C for 2 minutes, chilled on ice, and loaded onto a 8% polyacrylamide denaturing gel. Gels were run at 70 W for 2 hours. Autoradiographs of dried gels were on Amersham Hyperfilms (Amersham Life Science Inc., Arlington Heights, IL).

**In Vivo Studies**

Athymic Swiss nude mice, 10–12 weeks old (Charles River, Calco, Italy), were used throughout the study. The mice were maintained in laminar-flow rooms according to the U.K. Coordinating Committee on Cancer Research guidelines (15). Experimental protocols were approved by the Ethics Committee for Animal Experimentation of the National Cancer Institute (Milan). Each control
or drug-treated group included five mice bearing bilateral subcutaneous tumors. Tumor fragments (about $2 \times 2 \times 2$ mm) were implanted on day 0. Tumor growth was followed, and tumor volumes were calculated by the measurement of tumor diameters with a Vernier caliper and the use of the following formula: tumor volume ($TV = d^2 \times D/2$, where $d$ and $D$ are the shortest and the longest diameters, respectively. Drug treatments started when tumors were approximately 50 mm$^3$ in volume. Drugs were dissolved in sterile water for injection and injected intravenously. Details concerning treatment schedules are reported in the “Results and Discussion” section. The efficacy of the drug was assessed as tumor growth inhibition in drug-treated versus control mice and as $\log_{10}$ cell kill achieved by the drug treatment (see Fig. 3). A tumor was considered responsive when the drug treatment achieved a tumor volume inhibition greater than 60% and a $\log_{10}$ cell kill of 1 or more.

In experiments for determination of apoptotic indices, mice with MX-1 (breast carcinoma) and PVD (small-cell lung cancer) tumors were treated according to the therapeutic schedules of doxorubicin (every 7 days for 3 weeks) or MEN 10755 (every 3 or 4 days for five injections). On days 1, 3, 5, and 7 after the last drug treatment, tumors were excised from mice, immediately fixed in 4% buffered formaldehyde, and then embedded in paraffin. Paraffin sections (5 μm) were adhered to poly-L-lysine-pretreated slides. Tissue sections were then treated according to the method described by Gavielli et al. (16). For the TUNEL (i.e., terminal deoxynucleotide transferase-mediated digoxigenin-uridine triphosphate nick-end labeling) reaction, the In Situ Cell Death Detection POD Kit (Boehringer Mannheim, Ingelheim, Germany) was used. The final staining was done by use of a solution of diaminobenzidine (Sigma Chemical Co., St. Louis, MO). Sections were counterstained with hematoxylin. The apoptotic index was determined by using a light microscope at ×400 magnification and by recording the number of apoptotic nuclei per field.

**Statistical Analysis**

Tumor volumes in doxorubicin- and MEN 10755-treated mice were compared by use of Student’s $t$ test (two-tailed) to obtain $P$ values.

**Results and Discussion**

NMR analysis of MEN 10755 showed that the shape of the molecule up to the second sugar ring was similar to that of doxorubicin (17), but a major flexibility occurred at the glycosidic linkage between the fucose and daunosamine moieties. The geometry at the glycosidic bonds, defined by the angles $\phi = H(7)-C(7)-O(7)-C(1')$, $\phi' = H(4')-C(4')-O(4')-C(1')$, $\psi = C(7)-O(7)-C(1')-H(1')$, and $\psi' = C(4')-O(4')-C(1')-H(1')$, was studied with the assistance of NOE and $^3$J$_{CH}$ data. The methodology of NMR analysis of molecular flexibility in solution (18) was used to calculate feasible domain and corresponding matching functions of the corresponding molar fraction of 54 theoretical conformers. As a result, geometry about the $\phi$, $\psi$ linkages was well defined (values of about 42° and -15°, respectively), whereas for $\phi'$ and $\psi'$ the data could be satisfactorily explained by considering conformers with $\phi' = 49°$ and $\psi' = 6°$, but conformers with $\phi' = 89°$ and $\psi' = 24°$ could be present as a molar fraction up to 0.6 with a good degree of matching. This observation indicates a higher flexibility for the spatial arrangement of the second sugar ring. The most populated conformer (75%) in solution derived from our study is shown in Fig. 1.

The ability of the drug to stimulate topo-isomerase II-mediated DNA cleavage was investigated by the incubation of 5'-32P-labeled fragments of SV40 DNA with purified enzyme and different drug concentrations and by the analysis of DNA cleavage-intensity patterns with polyacrylamide denaturing gels (Fig. 2). Although both drugs stimulated similar DNA cleavage-intensity patterns, MEN 10755 was found to be more effective than doxorubicin in stimulating DNA cleavage. Such an ability was consistent with protein-associated DNA breaks induced in A2780 ovarian carcinoma cells. Indeed, a lower intracellular content of MEN 10755 caused a comparable extent of DNA lesions, thus suggesting an increased drug potency at the target level (Table 1). A reduced drug uptake might be related to the presence of an additional sugar moiety in the disaccharide, thus influencing the cytotoxic properties. This behavior is in contrast with the observation that, in the natural monoglycosides, removal of the methoxy group markedly enhances both cellular uptake and cytotoxic potency (1,4).

In spite of an apparently reduced cellular accumulation, MEN 10755 exhibited a striking antitumor activity in all preclinical models examined, including tumor systems relatively refractory to doxorubicin treatment (19,20). As a first step for in vivo evaluation, a study was performed in tumor-bearing mice to define optimal doses and schedules for each drug. Weekly treatment with 7 mg/kg doxorubicin for 3 weeks and treatment every 3 or 4 days for five injections with MEN 10755 should be considered as optimal, since both drugs caused death due to toxicity in one mouse of 32 mice treated. In comparison, a more intensive treatment was more toxic for doxorubicin, and a weekly schedule was less effective for MEN 10755. Fig. 3, A, compares the effects of optimal regimens of doxorubicin and MEN 10755 in the treatment of a human ovarian carcinoma (A2780), a breast carcinoma (MX-1), and a small-cell lung cancer (POVD). With the excep-
tion of the A2780 tumor, which was responsive to doxorubicin, the other tumors were refractory to doxorubicin. The molecular basis of the intrinsic resistance to doxorubicin in these systems was not related to overexpression of transport systems, such as the mdr-1 gene or Mrp (i.e., multidrug resistance-associated protein) [7; Zunino F: manuscript in preparation]. Under optimal conditions for each drug, the disaccharide analogue was superior to doxorubicin both in inhibiting tumor growth and in inducing partial regression of established tumors. Evidence of a therapeutic benefit of the treatment with MEN 10755 was clearly strengthened by a marked increase in log10 cell kill (Fig. 3, A). Indeed, a log10 cell kill value of greater than 1 was achieved by

Fig. 3. A) In vivo response of human tumor xenografts after treatment with intravenous doxorubicin or MEN 10755. Abscissa: days after tumor implantation. Ordinate: means of tumor volumes in mm3. Arrows indicate the first day and the last day of treatment. ○, control; ▲, doxorubicin (7 mg/kg, every 7 days for 3 weeks); ■, MEN 10755 (6 mg/kg, every 3 or 4 days for five injections). Tumor volumes 7 days after the last treatment statistically differed (by Student’s t test) between doxorubicin- and MEN 10755-treated mice: tumor volume inhibition, 94% versus 99% (P<.05) for A2780 ovarian tumor; 56% versus 96% (P<.0001) for MX-1 breast carcinoma; 44% versus 91% (P<.005) for POVD small-cell lung cancer. 

Table 1. Comparison of cellular accumulation and DNA single-strand breaks (DNA-SSB) induced by doxorubicin or MEN 10755 in A2780 human ovarian carcinoma cells

<table>
<thead>
<tr>
<th>Drug*</th>
<th>Extracellular concentration, μM</th>
<th>Uptake,† pmol/106 cells, mean ± standard deviation</th>
<th>DNA-SSB,‡ rad-equivalents, mean ± standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>1</td>
<td>51 ± 14</td>
<td>210 ± 141</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>384 ± 50</td>
<td>343 ± 127</td>
</tr>
<tr>
<td>MEN 10755</td>
<td>1</td>
<td>27 ± 7</td>
<td>230 ± 43</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>159 ± 50</td>
<td>367 ± 133</td>
</tr>
</tbody>
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*IC50 (i.e., drug concentrations required to inhibit cell growth by 50%) following 1-hour exposure, determined from dose–response curves, were 0.41 μM ± 0.2 μM and 0.39 μM ± 0.26 μM (means ± standard deviation) for doxorubicin and MEN 10755, respectively.

†Intracellular drug concentration expressed as pmol/10⁶ cells and quantified with respect to a calibration curve.

‡DNA-SSB were expressed as rad-equivalents by use of standard calibration curves obtained with cells exposed to γ rays.

B) Kinetics of apoptosis induction in MX-1 and POVD tumors treated as described in A. Apoptotic index was determined 1, 3, 5, and 7 days after the last drug treatment. For each time point, two tumors were examined. By use of a light microscope at ×400 magnification, the mean number of cells per field for each tumor model was assessed by the examination of 10 randomly chosen fields (i.e., 277 and 570 cells per field for MX-1 and POVD tumors, respectively). Ten fields of non-necrotic areas were selected in each section, in each field the number of apoptotic nuclei was recorded, and the apoptotic index is expressed as percentage of apoptotic nuclei relative to the mean number of cells per field. Mean values are reported. Standard error never exceeded 0.65. Empty bars: controls. Diagonal bars: doxorubicin. Diagonal crosshatch bars: MEN 10755.

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doxorubicin treatment only in the sensitive A2780 tumor, whereas the administration of MEN 10755 resulted in a pharmacologically relevant log_{10} cell kill in all tumors examined.

Although an increased activity of the doxorubicin analogue against sensitive tumors could be expected on the basis of enhanced drug interaction with the cellular target (i.e., topoisomerase II), the efficacy in the treatment of tumors with intrinsic resistance to doxorubicin was striking and is likely a result of several factors. It is noteworthy that the disaccharide analogue was very effective in stimulating the apoptotic response in human tumor cell lines (data not shown). Since intrinsic drug resistance (not mediated by transport systems) could reflect the inability of the drug to induce apoptosis (21), we investigated the in vivo induction of apoptosis in the MX-1 tumor after therapeutic treatment with doxorubicin or MEN 10755. Fig. 3, B, shows the time course of apoptosis in this tumor. The analogue induced a high level of apoptosis as early as 24 hours after the end of treatment, whereas doxorubicin exhibited no effect at that time and only a marginal increase in the apoptotic index later on. Thus, a plausible explanation for the increased efficacy of the disaccharide analogue is a cell-specific susceptibility to drug-induced early apoptosis, in relation to a drug-specific interaction with, or damage of, critical genomic sites (22), possibly as a result of the expanded recognition region in the molecule of the novel anthracycline compound. If this is the cellular basis for tumor responsiveness, a different pattern of tumor response would be expected for doxorubicin and MEN 10755. The critical events involved in activation of apoptosis by topoisomerase II inhibitors have not yet been identified. However, p53 status has been proposed as an important determinant of cellular response to DNA-damaging agents (21). Since MX-1 and A2780 tumors are characterized by wild-type p53, the efficacy of MEN 10755 treatment in these tumors is consistent with the ability of the cell to activate a p53-dependent pathway of apoptosis. If this interpretation is correct, the markedly different response of MX-1 to doxorubicin and its analogue suggests that wild-type p53 function is a necessary but not a sufficient condition for doxorubicin efficacy. In-deed, overexpression of Bcl-2 (data not shown), a well-known antiapoptotic protein involved in p53-dependent apoptosis, could account for the reduced susceptibility to apoptosis of MX-1 after doxorubicin treatment. The efficacy of the novel analogue suggests the ability of the drug to activate a p53-independent apoptosis.

The interpretation is consistent with a high activity of MEN 10755 against the PUVQ tumor. In fact, this small-cell lung cancer exhibited a mutant p53 (at codon 171, with GAG [glu] to TAG [stop] change), and this nonsense mutation is expected to lead to premature termination of protein, resulting in inactivation of p53 function. The efficacy of MEN 10755 treatment in a human lung tumor carrying a mutant p53 was consistent with the increased ability of the drug to induce p53-independent apoptosis, as supported by the pattern of apoptosis induction by equitoxic regimens of the two drugs (Fig. 3, B). Identification of new agents able to trigger p53-independent apoptosis is of pharmacologic relevance in human cancer therapy because loss of normal p53 function through mutation or deletion occurs in up to 50% of human tumors (23).

The differential biologic properties of MEN 10755 and doxorubicin, together with the high antitumor activity in a large series of human tumor xenografts including doxorubicin-resistant systems (19,20), make the disaccharide analogue a promising candidate for clinical evaluation.

References

(2) Weiss RB. The anthracyclines: will we ever find a better doxorubicin? Semin Oncol 1992; 19:670–86.


Notes
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