Anti-Cancer Drugs 1997, **8,** pp. 845–852

Cytotoxic and antitumor activity of MEN 10710, a novel alkylating derivative of distamycin

Mario Bigioni, Carmela Salvatore, Carla Palma, Stefano Manzini, Fabio Animati,[1] Paolo Lombardi,[1] Graziella Pratesi,[2] Rosanna Supino[2] and Franco Zunino[2]

Pharmacology and [1]Chemistry Departments, Menarini Ricerche, 00040 Pomezia (Rome), Italy. Tel: (+39) 6 91184503; Fax: (+39) 6 9100220. [2]Division of Experimental Oncology B, Istituto Nazionale per lo Studio e la Cura dei Tumori, 20133 Milan, Italy.

MEN 10710 is a new synthetic distamycin derivative possessing four pyrrole rings and a bis-(2-chloroethyl)-aminophenyl moiety linked to the oligopyrrole backbone by a flexible butanamido chain. Its biological properties have been investigated in comparison with the structurally related compound, tallimustine (FCE24517), and the classical alkylating agent, melphalan (L-PAM). Cytotoxic potency of MEN 10710 was increased from 10- to 100-fold, as compared to tallimustine or L-PAM in murine L1210, human LoVo and MCF7 tumor cell lines. MEN 10710 was still active against L1210/L-PAM leukemic cells, while a partial cross-resistance was observed in LoVo/DX and in MCF7/DX cells selected for resistance to doxorubicin and expressing a MDR phenotype. Treatment with verapamil (VRP) reduced the resistance to tallimustine, but not to MEN 10710, in MCF7/DX cells. The cytotoxic effects reflect *in vivo* antitumor potency and toxicity in the treatment of human tumor xenografts. MEN 10710 was more effective in A2780/DDP, an ovarian carcinoma selected for resistance to cisplatin. On the other hand, the IC_{30} for inhibiting murine granulocyte/macrophage colony formation was 50 times higher for MEN 10710 than for tallimustine, suggesting a lower myelotoxic potential. In conclusion, the particular biological profile of MEN 10710 characterized by a marked cytotoxic potency, an interesting antitumor efficacy and a reduced *in vitro* myelosuppressive action may represent a further improvement in the rational design of a novel distamycin-related alkylating compound.

Key words: Alkylating agent, distamycin, human tumor xenografts, myelotoxicity.

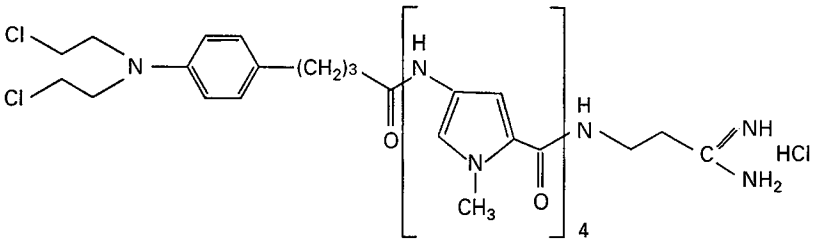
Introduction

Bifunctional alkylating agents are the first class of drugs proven to have clinical antitumor activity and still remain the most widely used drugs in clinical chemotherapy. Conventional nitrogen mustards are known to alkylate preferentially the nucleophilic N-7 atom of guanine in a non-random manner.[1] However, these agents have a limited capacity to read sequence information. In an attempt to increase the sequence selectivity of DNA damaging agents, a number of DNA minor-groove binders have been used as carriers of alkylating moieties.[2] Distamycin analogs appear the most promising compounds of this new series of antitumor agents. Among alkylating derivatives of distamycin, tallimustine (FCE 24517) exhibits a broad spectrum of antitumor activity against a variety of human tumor models, including tumor types (e.g. melanoma) resistant to conventional cytotoxic agents.[3] The antitumor activity of tallimustine has been related to a preferential binding to AT-rich sequences in the minor groove of B-DNA,[4,5] coupled to the alkylation of bases closely located to these DNA regions,[6] especially at N-3 of adenine.[7] At variance with L-PAM, tallimustine does not induce cross-linking.[8] Tallimustine is now undergoing clinical evaluation. However, preclinical and clinical data indicate a relevant myelosuppressive action.[9]

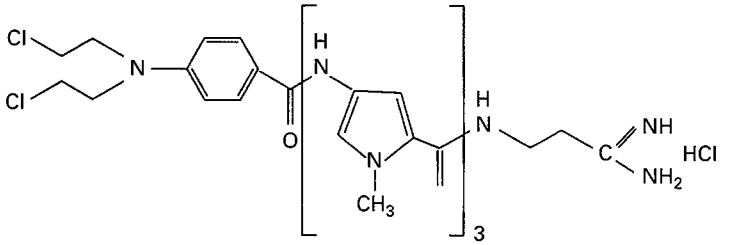
Recently, in our laboratories, a series of new compounds has been synthesized with the aim to examine the effect of the insertion of a flexible carbon chain between the oligopyrrole backbone and the alkylating moiety.[10] Among them MEN 10710 (Figure 1) emerged as a particular compound with an interesting profile of interaction with DNA different from both L-PAM and tallimustine. In fact it elicits alkylation at bases closely located to AT-rich tracts and in addition is a potent inducer of interstrand cross-links.[10] MEN 10710 is chemically characterized by two structural features: (i) the presence of four pyrrole rings, instead of three, in the distamycin backbone, and (ii) a butanamido chain as a linker between the oligopyrrole structure and the alkylating function.

This work was carried out in the frame of a joint project of A Menarini, Industrie Farmaceutiche Riunite, Florence and of Bristol-Myers Squibb Italia, Rome. It was supported by a grant of the Istituto Mobiliare Italiano (grant no. 53658).

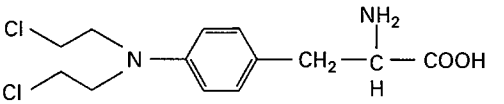
Correspondence to M Bigioni



MEN 10710



Tallimustine (FCE 24517)



L-PAM

Figure 1. Chemical structures of MEN 10710, tallimustine (FCE 24517) and L-PAM.

The aim of the present study was to perform a detailed comparative evaluation of MEN 10710, tallimustine and L-PAM in terms of cytotoxic, antitumor and *in vitro* myelosuppressive activity. Present findings indicate that MEN 10710 has a promising pharmacodynamic profile and it might represent an important step in the rationale design of new distamycin analogs with improved therapeutic index.

Materials and methods

Drugs

Tallimustine and its derivative MEN 10710 were obtained by chemical synthesis in the Chemistry Department, Menarini Ricerche, Pomezia (Italy). Their characterization and purity were assessed by comparing spectral data for tallimustine (FCE 24517) with those reported in the literature[3] and by NMR for the new compound. Both compounds were dissolved and diluted in deionized water. Doxorubicin obtained from Pharmacia-Upjohn (Milan, Italy) was diluted in deionized water and checked spectrophotometrically: $E_{1\%} = 200$ ($\lambda = 496$ nm, in H_2O). L-PAM (Alkeran®), purchased from Glaxo-Wellcome, was dissolved as described in the clinical formulation. Verapamil was purchased from Sigma (Milan, Italy), dissolved in dimethyl sulfoxide (DMSO) and diluted in deionized water. All drugs were prepared immediately before use.

Cell cultures and evaluation of cytotoxicity

L1210 and L1210/L-PAM murine leukemia cells were maintained in RPMI 1640 medium (Gibco) supplemented with 10% FBS, 2 mM L-glutamine and 0.01 mM β-mercaptoethanol. Human colon carcinoma LoVo (ATCC CCL 229) cell line was maintained in Ham's F12 supplemented with 10% FBS and vitamins. Human mammary carcinoma MCF7 (ATCC HTB 22) cell line was grown in Dulbecco's modification of Eagle's medium (DMEM), supplemented with 10% FBS and 2 mM L-Glutamine. LoVo/DX[11] and MCF7/DX[12] were obtained *in vitro* after treating LoVo and MCF7 cells with doxorubicin, and maintained in the presence of 0.2 and 1.0 μM of DX, respectively. One passage

before the experiment, the drug was removed from the culture medium.

Cell survival was assessed by the tetrazolium dye (MTT) assay.[13] The MTT dye was obtained from Sigma. Cells were harvested from exponential phase maintenance cultures and dispensed into 96-well microassay culture plates (3×10^3 cells/well) and grown for 24 h at 37°C in a 5% CO_2 incubator. Drugs were then added to the wells to achieve a final drug concentration ranging from 0.1 to 50 μM for L-PAM and from 0.001 to 10 μM for the others drugs (eight wells were used for each different concentration). The same volume of 0.9% NaCl solution in water with 1% (DMSO) was added to control wells. Wells containing culture medium alone without cells were used as blanks. The plates were incubated at 37°C in a 5% CO_2 incubator for 24 h. After drug exposure, cells were washed twice with 0.9% NaCl solution and incubated in drug-free medium for about three doubling times (72 h). When incubation was complete, 15 μl of stock solution of MTT dye in 0.9% NaCl solution in water was added to each well to achieve a final dye concentration of 0.5 mg/ml. The plates were incubated at 37°C in a 5% CO_2 incubator for 4 h. Subsequently, 100 μl of medium was removed from each well from the upper microwell layer and 100 μl of DMSO was added to solubilize the MTT formazan. Complete solubilization was achieved by placing the plate in a mechanical shaker for 30 min at room temperature. The optical density of each well was then measured with a Model 2550 Microplate Reader (BioRad) at a wavelength of 600 nm. The percent cell viability was calculated by the formula:

$$\text{Cell viability}(\%) = \frac{\text{mean optical density of treated wells}}{\text{mean optical density of control wells}} \times 100$$

The results were espressed as IC_{50}, calculated on dose-response curves, and defined as the drug concentration that produces 50% reduction of absorbance compared to control cells. Each experiment was repeated at least three times.

In a separate set of experiments ($n = 3$) the cytotoxicity of verapamil (VRP) on MCF-7 and MCF-7/DX cell lines was assessed. Verapamil was evaluated in the concentration range 0.1–100 μM (eight wells were used for each different concentration). At the highest non-cytotoxic concentration of VRP, corresponding to 55 μM, there was a cell survival of 89 ± 7 and $93 \pm 5\%$ in MCF-7 and in MCF-7/DX, respectively, and this value was not statistically different from the survival rate of cells exposed to a drug-free medium. Therefore this concentration of VRP was selected for following studies of co-incubation with cytotoxic drugs to assess any potential reversal of resistance.

Tumor lines and *in vivo* studies

First-generation hybrid BDF1 mice, 6–12 weeks old, were purchased from Charles River (Calco, Italy). In experiments with human tumor xenografts, Swiss *nu/nu* mice, 6–9 weeks old (Charles River), were maintained in microisolator cages and supplied with sterile materials.

A431 human epidermoid carcinoma (ATCC CRL 1555), H460 human non-small cell lung carcinoma (ATCC HTB 177) and A2780/DDP human ovarian carcinoma cisplatin-resistant (selected in Istituto Nazionale Tumori Laboratory by continuous drug exposure *in vitro* of the parent cisplatin-sensitive A2780 line; the resistance index to cisplatin is around 20) were established as s.c. *in vivo* lines from cell lines (10^7 cells/0.2 ml/flank). For experimental purposes mice were transplanted in both flanks with tumor fragments. Tumor-bearing mice were randomly divided into different groups before starting drug treatment. The s.c. tumors were measured in two diameters and the tumor weight (TW) was calculated by the formula: TW = length (mm) × width^2 (mm)/2 = mm^3 = mg (assuming unit density).[14]

The following end points were used to assess antitumor efficacy:

(i) Optimal tumor weight inhibition percent (opTWI%) in treated over control mice. Tumor weight (TW), calculated as described above, was the mean of each group. TWI% was evaluated as: 100 − (TW treated /TW control × 100). The lowest value in the first 30 days after the end of treatment was reported in the results.

(ii) Log_{10} cell kill (LCK) was calculated by the formula:[15]

$$\text{LCK} = \frac{T - C}{3.32 \times T_d}$$

$T - C$, is the tumor growth delay, where T is the time (days) required for the treated tumors and C is the control tumors (mean of eight tumors/group) to reach a predetermined size, usually 1 or 2 g. T_d is the doubling time in days measured from a best fit straight line of the control tumors in exponential growth (range 100–400 mg). A LCK value of 1 is considered indicative of an active compound.

(iii) T/C % in survival time, where T and C represent the median survival time (days) of treated and control mice, respectively.

Death of treated mice occurring prior to the first death in the control were ascribed to drug toxicity.

Student's *t*-test (two tailed) was used for statistical comparison of tumor weight in mice treated with MEN 10710- versus tallimustine-treated mice, at the days on which opTWI% was assessed.

Granulocyte macrophage colony-forming cell formation (GM-CFC) assay

GM-CFC assay conditions were based on the procedure of Volpe *et al.*[16] In brief, nucleated bone marrow cells were recovered from the femurs of female BDF1 mice, 6–12 weeks of age. Cells were suspended at a concentration of 2×10^6/ml in RPMI 1640 containing 20% heat-inactivated (65°C, 30 min) FBS with 5 mM HEPES, 2 mM L-glutamine, 100 U/ml of penicillin, 100 μg/ml of streptomycin and 5×10^5 mM 2-mercaptoethanol (Gibco), and incubated with or without various concentration of drugs ranging from 0.005 to 500 nM (see Figure 2). Following 4 h of incubation at 37°C with 5% CO_2 in a humidified atmosphere, the cells were centrifuged and extensively washed in RPMI 1640. Finally, 2×10^5 marrow cells were plated in triplicate 35 mm plastic Petri dishes, in 2 ml of complete RPMI 1640 medium containing 0.35% agarose (Life Technologies, Gaithersburg, MD) and 100 U/ml of murine recombinant granulocyte-macrophage colony stimulating factor (GM-CSF) (Genzyme, Cambridge, MA). The cultures were allowed to gel for 15 min at 4°C and then incubated for 7 days. GM-CFC colonies were identified as having 50 or more cells and scored by using a Leitz inverted microscope. The toxicity of drugs on colony formation was determined by comparing the mean number of colonies in treated groups (*T*) to that in untreated controls (*C*) according to the formula:

$$\text{Colony inhibition (\%)} = \frac{C - T}{C} \times 100$$

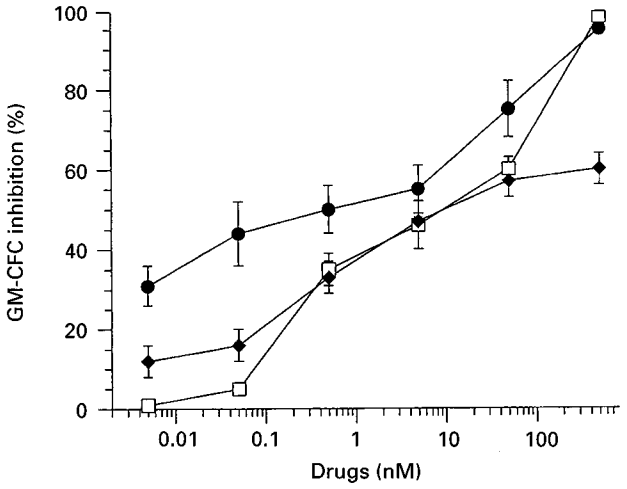


Figure 2. Effects of tallimustine, MEN 10710 and L-PAM on GM-CSF-dependent murine bone marrow colony formation. Nucleated bone marrow cells recovered from femurs of BDF1 mice were treated for 4 h at different concentrations with: tallimustine (●); MEN 10710 (□) and L-PAM (◆). Mean ± SE.

Results

Cytotoxic activity

The cytotoxic potency of MEN 10710 was 4- to 17-fold greater than tallimustine in human (LoVo and MCF7) and murine (L1210) cell lines (Table 1). In the same cell lines, L-PAM exhibited a mild cytotoxicity about two order of magnitude lower than MEN 10710. In L-PAM-resistant leukemia cells (L1210/L-PAM) MEN 10710 still exerted a remarkable cytotoxic action. On the other hand human cell lines selected for resistance to doxorubicin, LoVo/DX and MCF7/DX, largely characterized by a typical MDR phenotype,[11,12] were partially resistant to the growth inhibitory effects of both MEN 10710 and tallimustine. However, the pattern of cross-resistance of the two alkylating distamycin derivatives was different in the cell systems expressing *mdr 1*. In particular a high degree of resistance to tallimustine but not to MEN 10710 was observed in MCF7/DX. In this cell line further experiments were performed to examine the potential reversal of resistance to those agents by verapamil VRP. At its subtoxic concentration (55 μM) VRP considerably increased the cytotoxic effect of tallimustine (resistance index was reduced from 33 to 2) as well as of DX (resistance index dropped from 268 to 15), while leaving unaffected those of MEN 10710 (resistance index was 4.9 and 5.0 in the absence and presence of VRP, respectively).

Antitumor activity

The antitumor efficacy of the two distamycin derivatives was investigated in three human carcinoma xenografts characterized by natural resistance to alkylating agents such as the A431 cervix carcinoma[17] and the H460 non-small cell lung carcinoma,[18] or A2780/DDP an ovarian carcinoma with acquired resistance to cisplatin (see Materials and methods). The comparison of antitumor activity was performed

Table 1. Pattern of cytotoxicity and cross-resistance of distamycin derivatives, L-PAM and doxorubicin

Compound	IC_{50} (μM)		RI[a]	IC_{50} (μM)		RI[a]	IC_{50} (μM)		RI[a]
	L1210	L1210/L-PAM		LoVo	LoVo/DX		MCF7	MCF7/DX	
MEN 10710	0.014 ± 0.004[b]	0.018 ± 0.011	1.3	0.059 ± 0.02	0.248 ± 0.12	4.2	0.016 ± 0.01	0.079 ± 0.02	4.9 (5.0)
Tallimustine	0.248 ± 0.148	0.422 ± 0.031	1.7	0.560 ± 0.18	2.974 ± 1.10	5.3	0.080 ± 0.06	2.701 ± 2.06	33.8 (2.2)
L-PAM	2.900 ± 0.3	17.596 ± 0.7	6.1	2.860 ± 0.6	3.162 ± 0.76	1.1	2.830 ± 0.74	5.167 ± 0.15	1.8 (2.1)
DX	0.003 ± 0.001	0.004 ± 0.001	1.3	0.070 ± 0.02	4.467 ± 0.50	63.8	0.015 ± 0.01	4.026 ± 1.38	268.4 (15.6)

[a]Resistance index = IC_{50} on resistant cells/IC_{50} on sensitive cells. The RI values in the presence of VRP (55 μM) are given in parentheses.
[b]Mean of at least three experiments ± SD.

at doses selected in preliminary experiments and corresponding to their maximal tolerated doses according to two treatment schedules (Table 2). Mice body weight loss never exceeded 8% in all treated groups. MEN 10710 inhibited the growth of A431 with a TWI of 67% and caused a LCK of 1.1. The antitumor activities of MEN 10710 and tallimustine were comparable even on survival time, although MEN 10710 was effective at a lower dose level. A marginal antitumor response was obtained with both distamycin derivatives against the H460 tumor with a TWI around 50% and a LCK not exceeding 0.6. A remarkable antitumor activity was obtained with MEN 10710 treatment on A2780/DDP, a tumor line unresponsive to cisplatin, with 78% of TWI compared to 62% in tallimustine-treated mice ($p < 0.05$) and a remarkable value of LCK of 1.8 compared to 1.2 obtained with tallimustine. Moreover, the superior activity of MEN 10710 over tallimustine was evident also in terms of increased mice survival time (Table 2).

In vitro myelotoxicity of MEN 10710, tallimustine and melphalan

To evaluate the myelosuppression induced by anticancer drugs we studied the toxic effects on murine granulocyte-macrophage progenitors using an *in vitro* GM-CFC assay. As presented in Table 3 and in the Figure 2 all the drugs studied inhibited the GM-CFC formation in a concentration-related manner. Tallimustine and MEN 10710 were highly toxic versus murine myeloid precursors and approximately 300 nM of these agents were able to produce a complete suppression of colony formation. However, the IC_{30} and IC_{50} values were higher for MEN 10710 than for tallimustine: in fact, while at 0.006 nM tallimustine already determined a 30% inhibition of GM-CFC formation, MEN 10710 at this dose was still devoid of any significant suppressive effect and this level of toxicity was achieved with a dose 50 times higher (0.3 nM). L-PAM exerted the same myelotoxic effect at doses as 0.4 nM (IC_{30}), although at the maximal dose tested its inhibitory effect did not reach the IC_{90}.

Table 4 shows the ratios between the IC_{50} values in the myelotoxicity assay and the IC_{50} values against tumor cell lines obtained with the three compounds. MEN 10710 exhibited the most favorable ratio.

Discussion

The present study indicates that MEN 10710, a novel distamycin derivative, had an interesting pharmaco-

Table 2. Antitumor activity of MEN 10710 and tallimustine against human tumor xenografts of different tumor types

Tumor line	Compound	Route and treatment schedule	Dose (mg/kg/day)	opTWI[a] (%)	LCK[b] (g)	*T/C*%[c]	Toxic death/ total no. of mice
A431	MEN 10710	i.v., q4d×3	0.25	67	1.1 (1)	150	0/5
	tallismustine	i.v., q4d×3	1	68	1.1 (1)	144	0/5
	L-PAM	i.v., q4d×3	6	54	0.8 (1)	122	1/5
H460	MEN 10710	i.v., q7d×3	0.4	50	0.6 (1)	ND	0/5
	tallimustine	i.v., q7d×3	1	54	0.6 (1)	ND	0/5
	L-PAM	i.v., q7d×3	6	48	0.4 (1)	ND	0/5
A2780/DDP	MEN 10710	i.v., q7d×3	0.5	78[d]	1.8 (2)	226	0/5
	tallimustine	i.v., q7d×3	1	62	1.2 (2)	151	0/5
	cisplatin	i.v., q7d×3	6	40	0.3 (2)	93	0/5

[a]Optimal percentage of tumor growth inhibition, as the lowest value in 30 days after the last treatment.
[b]Log_{10} cell kill produced by the drug and calculated to the tumor weight reported in parentheses.
[c] Median survival time of treated/control mice × 100. A431 control: 32 days; A2780/DDP control: 31 days. ND = not determined because control mice did not die in the experimental time.
[d]$p < 0.05$ as compared to tallimustine in the same experiment.

Table 3. Comparison of tallimustine, MEN 10710 and L-PAM inhibition of GM-CSF-dependent murine bone marrow colony formation

Compound	IC_{30}[a] (nM)	IC_{50}[a] (nM)	IC_{90}[a] (nM)
Tallimustine	0.006±0.005 (n=4)	0.6±0.3 (n=4)	271±12 (n=4)
MEN 10710	0.3±0.1 (n=4)	7±2 (n=4)	258±8 (n=4)
L-PAM	0.4 (n=4)	14 (n=4)	ND (n=4)

[a]Each value is the mean±SE. Number of experiments is shown in parentheses.
ND = not determined.

Table 4. Evaluation of the therapeutic index of alkylating agents from *in vitro* results

Compound	IC_{50} bone marrow (nM)	IC_{50} tumor cells[a] (nM)	R^b
MEN 10710	7	30	0.23
Tallimustine	0.6	296	0.002
L-PAM	14	2863	0.005

[a]The numbers represent the average of the IC_{50} obtained on L1210, LoVo and MCF7 cells (see Table 1).
[b]Ratio between IC_{50} on bone marrow/IC_{50} on tumor cells.

dynamic profile characterized by a potent cytotoxic action, a significant antitumor activity and a reduced (as compared to tallimustine) *in vitro* myelotoxicity. Previous studies have indicated that unlike the parent compound tallimustine (but similar to the classical alkylating agent L-PAM), MEN 10710 is a strong inducer of inter-strand DNA cross-links.[10] Further, like tallimustine, it alkylates bases placed in close proximity of AT-rich regions and not at N-7 of guanine as conventional alkylating agents do. Therefore, MEN 10710 is endowed with a particular mode of interaction with DNA that might result in a different profile of preclinical activity. It is interesting to note that a similar profile of DNA interaction was observed with a derivative of MEN 10710 characterized by the presence of three pyrroles and the same butanamido chain.[10] Therefore, the peculiar pharmacological behavior appears to be related to the insertion of the aliphatic chain, thus widening the distance between pyrrole groups (probably recognizing the AT-rich regions) and the alkylating moiety that could be more reactive, thus favoring cross-linking ability. These molecular and biological features of MEN 10710 were associated with an enhanced cytotoxicity. Both on murine leukemia (L1210) and human carcinoma cell lines (LoVo and MCF7) MEN 10710 was one to two orders of magnitude more potent than reference compounds, i.e. tallimustine and L-PAM, respectively. Both MEN 10710 and tallimustine were effective in L1210/L-PAM cell lines, suggesting that the resistance mechanism(s) to classical alkylating agents operating in this cell line were not critical determinants of cell response to minor-groove alkylating agents.

Interestingly, a reduction in the cytotoxicity of distamycin derivatives was noted in two cell lines selected for DXR resistance, LoVo/DX and MCF-7/DX. In both lines an important mechanism of resistance is the classical MDR[11,12] and we have confirmed the expression of the gp170 protein in our lines by means of FACS analysis (data not shown). In the MCF-7/DX

cell line a substantial lower resistance index was obtained for MEN 10710 as compared to tallimustine. Previous studies indicated that tallimustine is cross-resistant in cell lines exhibiting the MDR phenotype,[19] suggesting that minor-groove binders containing the oligopyrrole chain could be recognized by a gp170-mediated extrusion mechanism. An indirect confirmation of this hypothesis was the observation that, in the presence of the MDR modulator VRP,[20] the cytotoxicity of tallimustine was significantly recovered in the MCF7/DX cell line. On the other hand, VRP failed to affect the cytotoxic action of MEN 10710, suggesting that, for this compound, the observed cross-resistance might be only marginally mediated by this transport system. It should be noted that in the MCF7/DX cell line other resistance mechanisms, in addition to *mdr 1* overexpression, have been proposed.[12,21] In particular an increase in glutathione-dependent detoxification mechanism(s) has been described in the resistant cell lines and this could concurr to cross-resistance against DXR and alkylating agents.[22] These other resistance mechanisms (VRP insensitive) could be particularly relevant for eliciting resistance to MEN 10710.

Regarding the antineoplastic activity on human tumor xenografts, MEN 10710 was partially effective in inhibiting the growth of the human epidermoid carcinoma A431, with efficacy comparable to that of tallimustine. However, the effective dosage of MEN 10710 was 4-fold lower than that of tallimustine. Thus, the enhanced cytotoxicity of MEN 10710 was reflected in an increased *in vivo* potency. A low inhibition of tumor growth was observed on H460 lung cancer for both compounds, in keeping with previous results with tallimustine against other human lung tumor models.[3] MEN 10710 showed a remarkable antineoplastic activity on the ovarian tumor line A2780/DDP selected for resistance to cisplatin achieving a significantly greater tumor growth inhibition than other alkylating agents. The cellular basis of cisplatin resistance of this tumor remains unknown. However, the development of resistance has been related to an increased cell ability to repair drug-induced DNA lesions.[23] If this is a relevant mechanism, the lack of cross-resistance of MEN 10710 may have pharmacological implications for future drug design.

As expected on the basis of the marked antiproliferative activity, myelotoxicity is a major drawback of tallimustine, threatening its further clinical development.[9] *In vitro* studies on murine bone marrow colony formation are widely used as predictive models of *in vivo* myelotoxicity.[16] The present results indicated that the threshold for a myelotoxic effect (IC_{30}) was much higher for MEN 10710 than for tallimustine. However, at high doses both compounds completely suppressed colony formation. The ratios between the IC_{50} for cytotoxicity and for myelotoxicity were 0.002 and 0.23 for tallimustine and MEN 10710, respectively (Table 4). Therefore, although both compounds were more myelotoxic than cytotoxic, it is noteworthy that the behavior of MEN 10710 appeared to be much more favorable. However, myelosuppression is expected to be a relevant limitation for the clinical development of agents of this class.

Conclusion

MEN 10710, when compared to other typical (L-PAM) and atypical (tallimustine) alkylating agents, exhibited an enhanced cytotoxic activity both in sensitive and in resistant human tumor cell lines. Unlike tallimustine, its cytotoxic properties were unaffected by VRP, suggesting that this compound is not recognized by gp170. MEN 10710 exerted antitumoral activity with a potency greater than L-PAM and tallimustine. The antineoplastic activity was particularly relevant in A2780/DDP, a human ovarian carcinoma selected for resistance to cisplatin. Further, MEN 10710 exhibited a lower inhibition of GM-CSF-dependent murine bone marrow colony formation as compared to tallimustine. Based on a more favorable profile in terms of balance between antitumoral properties and *in vitro* myelotoxicity, MEN 10710 represents an important step in the rational design of new alkylating derivatives of distamycin.

Acknowledgments

We thank Gilberto Piovacari for technical assistance and Simona Bozzitelli for editorial help.

References

1. Warpehoski MA, Hurley LH. Sequence selectivity of DNA covalent modification. *Chem Res Toxicol* 1988; **1:** 315–33.
2. Zunino F, Animati F and Capranico G. DNA minor-groove binding drugs. *Current Pharmaceutical Design* 1995; **1:** 83–94.
3. Pezzoni G, Grandi M, Biasoli G, *et al.* Biological profile of FCE 24517, a novel benzoyl mustard analogue of distamycin-A. *Br J Cancer* 1991; **64:** 1047–50.
4. Kopka ML, Yoon C, Goodsell D, Pjura P, Dickerson RE. The molecular origin of DNA-drug specificity in netropsin and distamycin. *Proc Natl Acad Sci USA* 1985; **82:** 1376–80.
5. Zimmer C, Wahnert V. Nonintercalating DNA-binding

ligands: specificity of the interaction and their use as tools in biophysical, biochemical and biological investigation of the genetic material. *Prog Biophys Mol Biol* 1986; **47:** 31.
6. Broggini M, Erba E, Ponti M, *et al.* Selective interaction of the novel distamycin derivative FCE 24517. *Cancer Res* 1991; **51:** 199-204.
7. Broggini M, Coley HM, Mongelli N, *et al.* DNA sequence-specific adenine alkylation by the novel antitumor drug tallimustine (FCE 24517), a benzoyl nitrogen mustard derivative of distamycin. *Nucleic Acid Res* 1995; **23:** 81-7.
8. Coley HM, Mongelli N, D'Incalci M. The effects of a benzoic acid mustard derivative of distamycin A (FCE 24517) and related minor groove-binding distamycin analogues on the activity of major groove-binding alkilating agents. *Biochem Pharmacol* 1993; **45:** 619-26.
9. Sessa C, Pagani O, Zurlo MG, *et al.* Phase I study of the novel distamycin derivative tallimustine (FCE 24517). *Ann Oncol* 1994; **5:** 901-7
10. Ciucci A, Manzini S, Lombardi P, Arcamone F. Backbone and benzoyl mustard carrying mojety modifies DNA interactions of distamycin analogues. *Nucleic Acid Res* 1996; **24:** 311-5.
11. Grandi M, Geroni C, Giuliani FC. Isolation and characterization of human colon adenocarcinoma cell line resistant to doxorubicin. Short communication. *Br J Cancer* 1986; **54:** 515.
12. Cowan KH, Batist G, Tulpule A, Sinha B, Myers CE. Similar biochemical changes in human breast cancer cell and carcinogen-induced resistance to xenobiotics. *Proc Natl Acad Sci USA* 1986; **83:** 9328-32.
13. Alley MC, Scudiero DA, Monks A, *et al.* Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res* 1988; **48:** 589-601.
14. Geran RI, Greenberg NH, MacDonald MM, Schumacher AM, Abbott BJ. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. *Cancer Chemother Rep* 1972; **3:** 1.
15. Corbett TH, Roberts BJ, Trader MW, *et al.* Response of transplantable tumors of mice to antracenedione derivatives alone and in combination with clinically useful agents. *Cancer Treat Rep* 1982; **66:** 1187-200.
16. Volpe DA, De-Lin DU, Zurlo MG, Mongelli N, Murphy MJ. Comparative *in vitro* myelotoxicity of FCE 24517, a distamycin derivative, to human, canine and murine hematopoietic progenitor cells. *Invest New drugs* 1992; **10:** 255-61.
17. Fan Z, Baselga J, Masui H, Mendelsohn J. Antitumor effect of anti-epidermal growth factor receptor monoclonal antibodies plus *cis*-Diamminedichloroplatinnum on well established A431 cell xenografts. *Cancer Res* 1993; **53:** 4637-42.
18. Dykes DJ, Abbott BJ, Mayo JG, *et al.* Development of human tumor xenograft models for *in vivo* evaluation of new antitumor drugs. *Contrib Oncol* 1992; **42:** 1-22.
19. Capolongo L, Melegaro G, Broggini M, Mongelli N, Grandi M. Characterisation of LoVo subline resistant to a benzoyl mustard derivative of distamycin A (FCE 24517). Short communication. *Br J Cancer* 1993; **68:** 916-9.
20. Ford JM, Hait WN. Pharmacology of drugs that alter multidrug resistance in cancer. *Pharmacol Rev* 1990, **43:** 155-99.
21. Leonessa F, Jacobson M, Boyle B, Lippman J, McGarvey M, Clarke R. Effect of tamoxifen on the multidrug-resistant phenotype in human breast cancer cells: isobologram, drug accumulation and M_r 170,000 glycoprotein (gp 170) binding studies. *Cancer Res* 1994; **54:** 441-7.
22. Hamilton TC, Winker MA, Louie KG, *et al.* Augmentation of adriamycin, melphalan and cisplatin cytotoxicity in drug-resistant and -sensitive human ovarian carcinoma cell lines by buthionine sulfoximine mediated glutathione depletion. *Biochem Pharmacol* 1985, **14:** 2583-6.
23. Behrens BC, Hamilton TC, Masuda H, *et al.* Characterization of a *cis*-diamminedichloroplatinum(II)-resistant human ovarian cancer cell line and its use in evaluation of platinum analogues. *Cancer Res* 1987; **47:** 414-8.

(Received 6 May 1997; accepted 31 July 1997)