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ORIGINAL ARTICLE

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Synergistic effects of F 11782, a novel dual inhibitor of topoisomerases I and II, in combination with other anticancer agents

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Abstract *Purpose*: F 11782, or 2′,3′-bis-pentafluorophenoxyacetyl-4′,6′-ethylidine-β-D-glucoside of 4′-phosphate-4′-dimethylepipodophyllotoxin 2*N*-methyl glucamine salt, a novel dual catalytic inhibitor of topoisomerases I and II, characterized by marked antitumour activity in vivo in a series of experimental murine and human tumours, has been selected for further development. This preclinical study was undertaken to investigate its potential for inclusion in combination chemotherapy regimens. The in vitro cytotoxicity of F 11782 incubated simultaneously with the following drugs was investigated: aclarubicin, cisplatin, doxorubicin, etoposide, 5-fluorouracil, mitomycin C, paclitaxel, topotecan or vinorelbine. *Methods*: The combinations were first evaluated in vitro against the GCT27 human testicular teratoma cell line and then against the A549 human non-small cell lung cancer cell line using median effect analysis. *Results*: F 11782 in combination with cisplatin, mitomycin C, etoposide or doxorubicin showed synergistic cytotoxicity against both cell lines. Moreover, F 11782 combined with cisplatin or mitomycin C showed antitumour activity in vivo against P388 murine leukaemia grafted intravenously. Such synergy might have resulted from the identified nucleotide excision repair inhibitory activity of F 11782. *Conclusions*: F 11782 appears to be a promising candidate for combination chemotherapy, especially with DNA-damaging agents.

Keywords F 11782 · Combination index · Synergy · Cultured cells · In vivo

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Introduction

F 11782, the 2′,3′-bis pentafluorophenoxyacetyl-4′,6′-ethylidine-β-D-glucoside of the 4′-phosphate-4′-dimethylepipodophyllotoxin 2*N*-methyl glucamine salt [14], is a novel derivative of etoposide which has shown marked antitumour activity in vivo in a series of experimental tumour models [12, 23, 35]. In vitro studies have identified a mode of action different from that of other topoisomerase inhibitors, since F 11782 has been reported to show significant in vitro inhibitory activity against the catalytic activities of both topoisomerases I and II [28]. F 11782 appears to have a unique mode of interaction with these nuclear enzymes by preventing their interaction with DNA, yet without any detectable interaction with DNA itself [28], although the exact mechanism of action of F 11782, which leads ultimately to cell death, remains unclear [3, 4]. The extent of in vivo activity and its original mode of interaction with topoisomerases were major factors in the selection of F 11782 for clinical development.

Agents that inhibit topoisomerases I or II are among the most effective antineoplastic drugs currently available for cancer therapy. The successful clinical development of topoisomerase I poisons, essentially camptothecins, highlights the importance of topoisomerase I as a target for cancer therapy by demonstrating their efficacy against a wide range of cancers and importantly against metastatic colorectal and ovarian tumours [34]. Topoisomerase II-interacting epipodophyllotoxins (etoposide and teniposide) also show significant antitumour activity against a variety of neoplasms, including germ-cell malignancies, lung cancer, non-Hodgkin's lymphomas, leukaemias, Kaposi's sarcoma, neuroblastoma and soft-tissue sarcomas [15]. Such activity demonstrates the importance of topoisomerase II as an anticancer drug target. Nevertheless, all these compounds are 'poisons' of either topoisomerase I or II and until now, catalytic inhibitors of these nuclear enzymes remain as investigational agents not yet approved for clinical usage. In this regard, F 11782

as a novel dual catalytic inhibitor of these topoisomerases with its original mode of action, is of definite interest, and its clinical development has been initiated.

This preclinical study was therefore undertaken to investigate the in vitro cytotoxicity of F 11782 in combination with a series of other anticancer drugs selected on the basis of their widespread usage in cancer chemotherapy and their differing modes of action. They included topotecan and etoposide, poisons of, respectively, topoisomerases I and II [9], aclarubicin, a dual catalytic inhibitor of topoisomerases I and II [31, 32], 5-fluorouracil, an antimetabolite [19], paclitaxel and vinorelbine, two agents which, respectively, stabilize and destabilize tubulin polymerization [30], cisplatin and mitomycin C, two DNA crosslinking agents [29], and doxorubicin, a DNA intercalating agent [1]. Combinations were first evaluated against the GCT27 human testicular teratoma cell line, using median-effect analysis and calculating the combination index (CI) according to the method described by Chou and Talalay [7]. The results were then compared with those obtained testing similar combinations against A549 human non-small cell lung cancer (NSCLC) cells. Finally, complementary experiments focused on the synergistic effects identified when F 11782 was combined with cisplatin or mitomycin C, with the goal of confirming such activity using in vivo models and putting forward a molecular explanation of such synergy.

Materials and methods

Cell lines and cell culture

All cells were cultivated as monolayers in an incubator at 37°C under a humidified atmosphere of air containing 5% CO_2. Human GCT27 teratoma cells (gift from Dr. J.R.W. Masters, University College, London, UK) were cultivated in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS). A549 human NSCLC cells were obtained from the American Type Culture Collection (ATCC, Rockville, Md.) and were cultivated in minimal essential medium (MEM) supplemented with 10% heat-inactivated FCS. All cell culture media were supplemented with fungizone (final concentration 1.25 μg/ml), penicillin-streptomycin (final concentration 100 IU/100 μg/ml) and glutamine (final concentration 4 m*M*). MEM, FCS, fungizone and penicillin-streptomycin were purchased from Gibco (Cergy-Pontoise, France). RPMI-1640 medium, L-glutamine and trypsin-EDTA were purchased from Seromed (Polylabo, Strasbourg, France).

Mice and tumour model

Female hybrid CDF1 (CD2F1/CrlBR) mice (Charles River, St Aubin-les-Elbeuf, France) were used for implanting the murine P388 leukaemia (Division of Cancer Treatment, Tumour Repository, NCI, Frederick, Md.). Animals were handled and cared for in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and European Directive EEC/86/609, under the supervision of authorized investigators.

Chemicals and drugs

F 11782 and topotecan were synthesized at the Centre de Recherche Pierre Fabre (Castres, France). Vinorelbine ditartrate and etoposide were supplied by Pierre Fabre Medicament (Gaillac, France). [^{3}H]Thymidine (25 Ci/mmol) was purchased from Amersham (Saclay, France). All other compounds tested were purchased from Sigma (Saint-Quentin Fallavier, France). Vinorelbine, 5-fluorouracil, 5-fluoro-2′-deoxyuridine, mitomycin C, doxorubicin hydrochloride, hydroxyurea, mechlorethamine and topotecan were dissolved in water (final concentration 1%), cisplatin was dissolved in 0.9% sodium chloride solution (final concentration 1%), and aclarubicin, etoposide and paclitaxel were dissolved in DMSO (final concentration 0.1%).

Cell growth inhibition

For adherent cultures of GCT27 and A549 cells the drug-induced cytotoxic effects were evaluated using a 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as described previously [11]. GCT27 and A549 cells were inoculated into 96-well microtitre plates at, respectively, 5×10^4 and 2.5×10^4 cells/ml to ensure their logarithmic growth throughout the experiments. After allowing 24 h for cell attachment, fresh medium containing test compound either alone or in combination, or solvent at the required concentration was added to each well. GCT27 or A549 cells were placed in an incubator at 37°C under an atmosphere of air containing 5% CO_2, respectively, for 72 h or 48 h which corresponded approximately to two doubling-times of cell growth. Supernatants were then discarded and replaced with 0.1 ml of 1 mg/ml MTT solution in RPMI-1640 medium without phenol red and incubated for 3 h with MTT. Finally, 0.1 ml DMSO was added to each well to dissolve the formazan crystals formed, and the plates were read using a spectrophotometer (MR7000; Dynex Technologies, Issy les Moulineaux, France) at 570 nm (test wavelength) and 630 nm (reference wavelength). Irrespective of the cell line used, each condition tested was evaluated at least three times in three independent experiments in sextuplicate.

Combination index method

The combination effects of two drugs in terms of synergy, additivity or antagonism were analysed by the median effect plot [7], as detailed previously [2]. This method was selected because it takes account of the potency of each drug and their combinations and the shapes of their dose-effect curves. Initially the cytotoxicity of each compound alone was determined to simulate additive effect, then five dilutions of F 11782 combined with each test drug were performed. The experimental data were compared with the simulated curve corresponding to an additive effect using the CalcuSyn program (Biosoft, Cambridge, UK). This comparison enabled calculation of the CI which was plotted as a function of the fraction affected [7]. When $CI = 1$ the interaction is considered additive, when $CI < 1$ synergy is indicated and when $CI > 1$ antagonism is indicated. The computer-calculated CI value for 50% cytotoxicity ($fa = 0.5$), termed the CI_{50} value, was selected to define synergism, additivity or antagonism since its distorted error distribution, as a consequence of the linearization of the experimental data, is theoretically minimal [26]. Moreover, the envelope of additivity was arbitrarily enlarged to 0.8–1.2 since the original method does not take into account the precision of the data, as discussed by Greco et al. [13].

Unscheduled DNA synthesis

Detection of unscheduled DNA synthesis (UDS) was carried out according to the method of Bootsma et al. [6]. A cell suspension (3×10^4 cells/ml growth medium) was incubated on slides at 37°C in air containing 5% CO_2 for 18 h. Medium was then aspirated and, after a 4-h incubation at 37°C with 0.1 m*M* mechlorethamine, cells were incubated in growth medium containing F 11782, 10 μ*M* 5-fluoro-2′-deoxyuridine and 10 m*M* hydroxyurea to inhibit replication, and labelled with 10 μCi/ml [^{3}H]thymidine. Cells were washed three times for 20 min with growth medium containing

200 μ*M* non-radiolabelled thymidine, then fixed with methanol/acetic acid (3:1) for 15 min and air dried. Slides were dipped for 10 s into an autoradiographic emulsion (EM-1, Amersham). After 5 days exposure in the dark at 4°C, slides were developed (Kodak, Polylabo) and the nuclei were counterstained with Mayer's haemalum solution diluted 1:5 (Merck, Nogent sur Marne, France). Cells were viewed using a Zeiss Axioplan microscope combined with a KY-F50 camera (JVC) using a ×40 objective.

Experimental chemotherapy

All experiments were conducted in compliance with French regulations and CRPF ethical committee guidelines, based on the UKCCCR guidelines for the welfare of animals in experimental neoplasia, as detailed previously [21]. P388 cells were implanted intravenously (i.v.) into C2DF1 mice (10^6 cells/mouse) on day 0. After randomization into treatment cages on day 1, two test compounds were administered intraperitoneally (i.p.) as single doses, with the second compound of the combination given 30 min after the first. In each chemotherapy trial, mice were checked daily, and any adverse clinical reactions were noted and deaths recorded. Mice were weighed twice weekly during treatment and once weekly thereafter.

Evaluation of antitumour activity

Increase in life span (ILS) was calculated as a percentage (ILS%) as follows: [(median survival of treated mice/median survival of control mice)×100–100].

Results

Initially, the dose-effect relationships of each drug against GCT27 cells were subjected to the median effect plot to determine their potency (Dm); the results obtained are summarized in Table 1. The correlation coefficients (*r* values) were 0.95 or greater, indicating a good linear relationship and good reproducibility. All ten compounds were potent cytotoxic agents with Dm values ranging from 0.0091 to 6.2 μ*M*.

Combinations of F 11782 with each of the other drugs were first evaluated against GCT27 cells. Thus, cells were simultaneously treated with the two drugs for 72 h, then cytotoxicity was evaluated. Each CI determination was the result of at least three independent experiments containing at least five different dilutions of the two drugs. These dilutions were established as ratios of the Dm values indicated in Table 1. Thus, the combination ratio was designed to approximate the IC_{50} ratio of the component drugs, so that the contribution of the effect of each drug in the mixture would be about the same (i.e. equipotency ratio). Results corresponding to combinations of F 11782 with etoposide, topotecan and aclarubicin are shown in Fig. 1A, B and C, respectively. Each experimental point and the corresponding curves which indicate, for each fractional effect, the CI values (±1.96 SD) generated by the median effect analysis are shown. The lower the CI value, the higher the extent of synergy. Conversely the higher the CI value, the greater the antagonism, whilst additivity was established when the CI value was around 1, indicating that the experimental points were superimposable on those of the calculated curve of additivity. Thus, the three combinations exemplified in Fig. 1 correspond to synergism, additivity and antagonism, respectively. The overall results for each combination CI_{50} value are summarized in Fig. 2. Synergy was identified for the combinations of F 11782 with etoposide ($CI_{50}=0.53$), doxorubicin ($CI_{50}=0.56$), paclitaxel ($CI_{50}=0.50$), mitomycin C ($CI_{50}=0.70$) and cisplatin ($CI_{50}=0.74$). However, only additivity was identified for the combinations of F 11782 with topotecan ($CI_{50}=0.98$) and 5-fluorouracil ($CI_{50}=0.98$), whilst additivity or a slight synergism was noted for the combination of F 11782 with vinorelbine ($CI_{50}=0.80$). On the other hand, antagonism was identified for the combination of F 11782 with aclarubicin ($CI_{50}=1.47$).

All these data were obtained using GCT27 cells, and it is well known that the cytotoxicity of anticancer drugs varies depending on the cell line tested, and that such variation may in turn influence the effects of drug combinations [16, 17, 25, 27]. For this reason, median effect analysis was performed on similar drug combinations in A549 human NSCLC cells. The cytotoxicity of each compound tested alone for 48 h was determined and Dm values are indicated in Table 1. Again the correlation coefficients (*r* values) were 0.96 or greater, indicating a good linear relationship and thus validating the use of median effect analysis.

Table 1. Compounds tested in combination, their modes of action and their cytotoxicities against cultured GCT27 or A549 tumour cells. Dm values were calculated using CalcuSyn Software (Biosoft, UK) and correspond to the concentration (μ*M*) necessary to reduce proliferation of cells to 50% of that obtained with solvent only. Cells were incubated for 48 h in the presence of the compound and then cell viability was determined using an MTT assay as described in Materials and methods

Test compound	Mode of action	Inhibition of proliferation of (Dm ± SD; μ*M*)	
		GCT27 cells	A549 cells
F 11782	Topo I/II interaction	0.18 ± 0.03	67 ± 0.02
Aclarubicin	DNA interaction	0.0120 ± 0.005	0.045 ± 0.008
Cisplatin	DNA alkylation	1.7 ± 0.09	15 ± 1.2
Doxorubicin	DNA interaction	0.0091 ± 0.0012	0.49 ± 0.06
Etoposide	Topo II interaction	0.24 ± 0.04	7.7 ± 1.2
5-Fluorouracil	Antimetabolite	6.2 ± 1.2	33 ± 4.3
Mitomycin C	DNA alkylation	0.021 ± 0.003	1.4 ± 0.1
Paclitaxel	Tubulin interaction	0.053 ± 0.008	0.022 ± 0.003
Topotecan	Topo I interaction	0.12 ± 0.011	0.026 ± 0.0034
Vinorelbine	Tubulin interaction	0.011 ± 0.0018	0.016 ± 0.0021

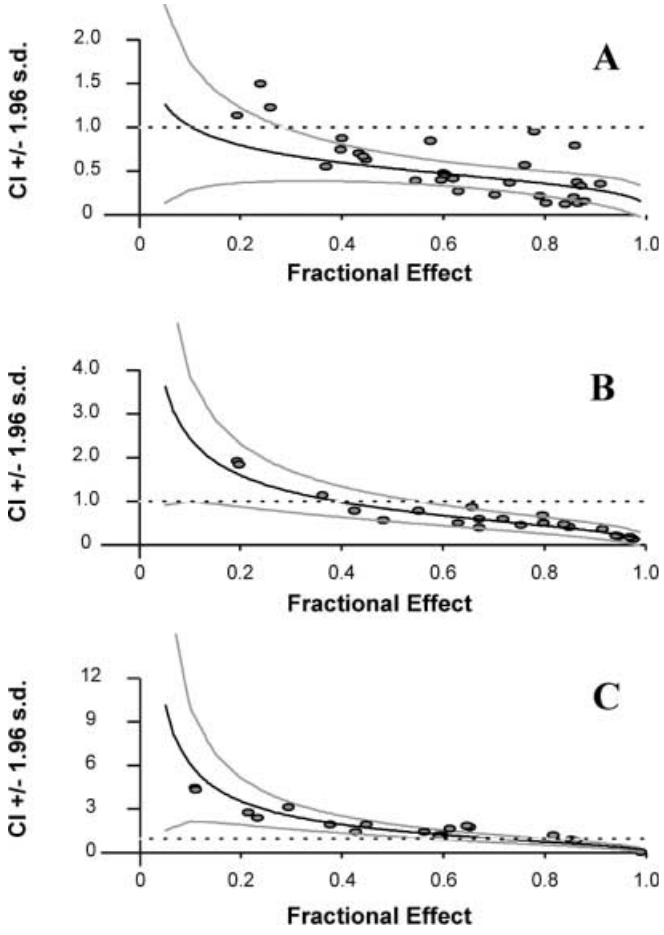

Fig. 1A–C. Data resulting from the combinations of F 11782 with etoposide (**A**), topotecan (**B**) and aclarubicin (**C**) in GCT27 tumour cells and analysed by the median effect analysis program (Biosoft, Cambridge, UK)

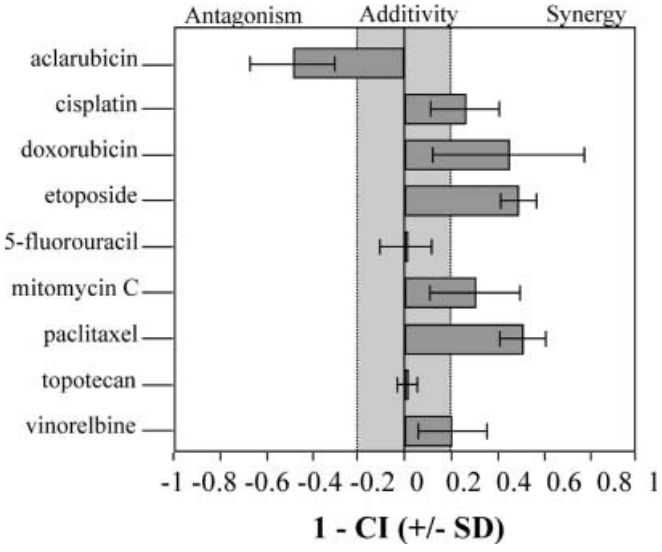

Fig. 2. Histogram summarizing the CI values calculated for 50% cytotoxicity (fa = 0.5) against GCT27 tumour cells treated with the combinations of F 11782 with each of the other nine drugs shown

Most of the combinations tested induced similar effects against both of these human tumour cell lines (Fig. 3). Clear synergism was found in A549 cells for the combinations of F 11782 with etoposide ($CI_{50} = 0.71$), doxorubicin ($CI_{50} = 0.79$), and either of the two DNA-damaging agents mitomycin C or cisplatin ($CI_{50} = 0.43$ and 0.34, respectively), whilst slight synergism was found for the combination of F 11782 with vinorelbine ($CI_{50} = 0.77$). Additivity was found for the combination of F 11782 with topotecan ($CI_{50} = 0.91$) and antagonism was found for the combination of F 11782 with aclarubicin ($CI_{50} = 4.1$). The only variation noted related to the combinations of F 11782 with either 5-fluorouracil or paclitaxel, since in A549 cells these combinations proved highly antagonistic ($CI_{50} = 3.9$ and 2.6, respectively). Nevertheless, overall, these data indicate that the general pattern of these combinations as synergistic, additive or antagonistic was similar in both cell types.

The levels of synergy for the combinations of F 11782 with either of the two DNA-crosslinking agents, cisplatin and mitomycin C, characterized by CI_{50} values of 0.24 and 0.36, respectively, were particularly high in A549 cells. On the other hand, a previous study has shown that F 11782, unlike other topoisomerase-interacting compounds, is a potent inhibitor of nucleotide excision repair (NER) in vitro [5]. Such inhibitory activity was confirmed in cultured A549 cells in terms of a decrease in UDS in the presence of F 11782 after treatment with mechlorethamine (Fig. 4). Thus, the number of silver grains representative of DNA repair in response to damage induced by 0.1 m*M* mechlorethamine (Fig. 4B) was dramatically reduced in the presence of 0.56 μ*M* F 11782 (Fig. 4C), and images corresponding to such treatment were similar to those of cells not treated with mechlorethamine (Fig. 4A). Moreover, this inhibitory activity depended on the concentration of F 11782 tested (data not shown) and was identified following exposure to cytotoxic concentrations of F 11782, suggesting that the inhibitory activity of F 11782 against NER may be taken into consideration in interpreting the synergism identified with either cisplatin or mitomycin C.

To determine whether such synergy could also be found in vivo, the effects of F 11782 combined with either cisplatin or mitomycin C, given as single i.p. administrations, in murine P388 leukaemia implanted i.v. were evaluated (Fig. 5). Administration of suboptimal doses of F 11782 (40 and 80 mg/kg) or cisplatin (2.5 mg/kg) alone increased the life span of mice bearing i.v.-implanted leukaemia, with ILS values of 43% and 100%, and 57%, respectively (Fig. 5A). Treatment with F 11782 and cisplatin in combination prolonged the life span of tumour-bearing mice with a clear dose effect, as compared with treatment with F 11782 or cisplatin alone (Fig. 5A). The maximal ILS was obtained with the combination of 80 mg/kg F 11782 with 2.5 mg/kg cisplatin, which gave an ILS value of 257% relative to 100% and 57% obtained with F 11782 and cisplatin alone, respectively (Fig. 5A). In addition,

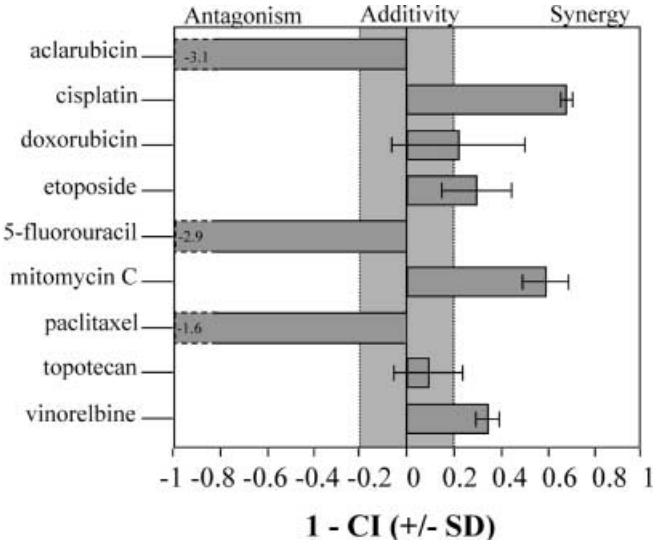

Fig. 3. Histogram summarizing the CI values calculated for 50% cytotoxicity (fa 0.5) against A549 tumour cells treated with the combinations of F 11782 with each of the other nine drugs shown

neither F 11782 nor cisplatin alone or in combination resulted in any toxicity as reflected by body weight change. Indeed, the maximal loss recorded was of 2.6% of the initial body weight for the combination of 80 mg/kg F 11782 and 2.5 mg/kg cisplatin, and so did not exceed 15%, the threshold of toxicity for this parameter according to NCI criteria [24].

F 11782 in combination with mitomycin C was also beneficial in vivo (Fig. 5B). Maximal effects were obtained with the combination of 80 mg/kg F 11782 with the suboptimal dose of mitomycin C of 5 mg/kg, as reflected by an ILS of 471%, relative to 129% and 100% obtained with F 11782 and mitomycin C alone, respectively (Fig. 5B). Furthermore, no additive effects with respect to host toxicity were noted with the combination of F 11782 with mitomycin C. Indeed, body weight changes recorded with F 11782 or mitomycin C alone were 0.5% to 2.1%, comparable to those of 2.8% and –1.6% for the combination treatments.

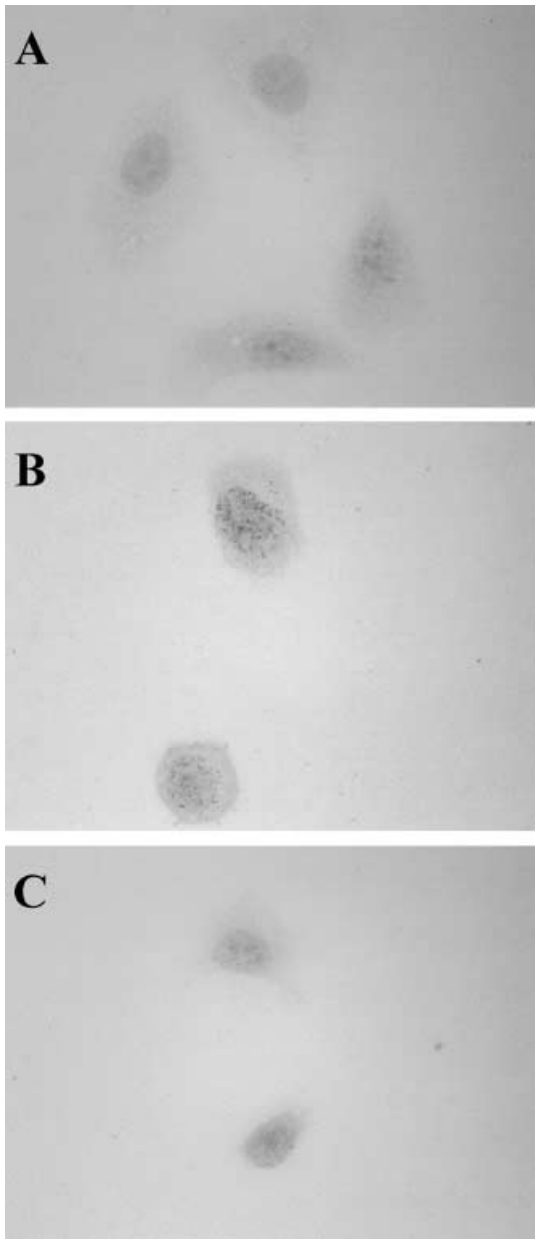

Fig. 4A–C. Autoradiographs showing the inhibition by F 11782 of unscheduled DNA synthesis (UDS) in A 549 cells. Cells were incubated with medium only (**A**) or treated with 0.1 m*M* mechlorethamine only (**B**) or with 0.1 m*M* mechlorethamine in the presence of 0.056 μ*M* F 11782 (**C**)

Discussion

Overall, moderate or high levels of synergy were found for combinations of F 11782 with antitumour compounds with completely different modes of action. Synergy was identified in cultured GCT27 and A549 tumour cells for combinations of F 11782 with either cisplatin or mitomycin C, two DNA-damaging agents, with doxorubicin, a DNA-intercalating agent, and with etoposide, a topoisomerase II poison. Moderate synergy or additivity was also identified for the combination of F 11782 with vinorelbine, a microtubule-destabilizing agent, and a strict additivity was found for the combination of F 11782 with topotecan, a topoisomerase I poison. These findings suggest broad possibilities for including F 11782 in combination chemotherapy regimens. On the other hand, only rare cases of antagonism were identified with combinations including F 11782. Such antagonisms might be important caveats for the clinical use of F 11782 in combination, but these in vitro data have to be considered as an initial approach to drug combinations and need to be confirmed in vivo. Nevertheless, overall these data suggest that F 11782 might usefully be combined with a large panel of anticancer drugs.

It has been demonstrated in several previous studies that the effects of combinations of antitumour drugs may vary depending on the tumour cell line studied [16, 17, 25,

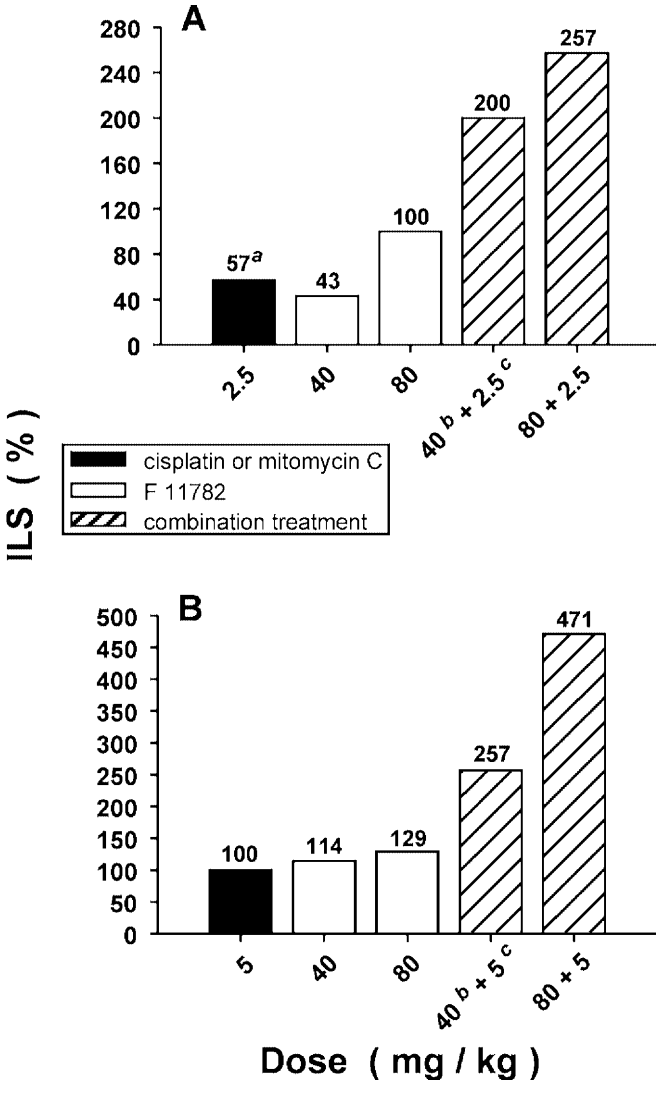

Fig. 5A, B. Combination effects of F 11782 plus cisplatin (**A**) or mitomycin C (**B**) against the murine P388 leukemia implanted i.v. into CDF1 mice. On day 0, 10^6 P388 cells were inoculated i.v. into mice, and i.p. treatments were given on day 1, with cisplatin or mitomycin C administered 30 min after F 11782. [a]ILS value, where ILS (%) = [(median survival of treated mice/median survival of control mice)×100–100], and according to NCI standard criteria for the P388 tumor model, ILS 20–75% is the minimal range for activity [36]. [b,c]Dose (mg/kg) of the first compound (F 11782) and the second compound, cisplatin (**A**) or mitomycin C (**B**), given in the combination

27]. In this study, synergy identified with combinations of F 11782 with cisplatin, doxorubicin, etoposide or mitomycin C in GCT27 human teratoma cells was also noted in A549 human NSCLC cells. These experiments could be extended to other cell lines to evaluate more fully the potential of such combinations against different types of tumour cells. However, our data provide evidence that such synergism is not restricted to a single tumour cell type and provide background information vis-à-vis the potential value of F 11782.

Interestingly, a supra-additive effect of the combination of F 11782 with etoposide has also been demonstrated previously using murine leukaemia L1210 cells [12]. The fact that synergistic effects have been identified with the combination of F 11782 and etoposide, irrespective of the cell line tested, reinforces the fact that these two epipodophylloid derivatives have a quite different mode of action. On the other hand, the antagonism identified in both cell lines with the combination of F 11782 and aclarubicin, another dual inhibitor of topoisomerases [31, 32], tends to confirm that the cytotoxicity of F 11782 is indeed related to its inhibitory activity against topoisomerases I and II.

The only variation noted between the results obtained using the two tumour cell lines A549 and GCT27 relates to combinations of F 11782 with 5-fluorouracil or paclitaxel. Such a variation might have resulted from differences in the genetic background of the two cell lines tested. However, it has already been shown in previous studies that certain topoisomerase inhibitors, such as etoposide, topotecan and SN38, when combined with paclitaxel result in an antagonistic effect against A549 NSCLC cells [10, 17, 18, 25, 27], whilst such combinations are either additive or synergistic against teratocarcinoma cell lines [8]. Pharmacogenomic studies might assist in our understanding of such phenomena by establishing in each cell line the pattern of protein expression in response to each drug.

Particularly potent in vitro synergism against A549 cells was identified with the DNA-damaging agents, cisplatin and mitomycin C. Interestingly, F 11782 proved an effective inhibitor of NER in these A549 cells, and such inhibitory activity might well explain, at least in part, the synergistic effects observed for combinations of F 11782 with either cisplatin or mitomycin C. On the other hand, only a low level of synergy was found for combinations of F 11782 with cisplatin or mitomycin C using GCT27 cells. A recent study, however, has demonstrated that cells from the testicular tumour cell lines GCT27 and 833 K are deficient in their repair of cisplatin-induced DNA damage, and this defect results from a reduced level of XPA protein [20]. Such defective DNA repair was confirmed in our study by the detection of only a low level of UDS in GCT27 cells (data not shown). Moreover, this level of UDS was too low to show a significant effect of F 11782 on DNA repair in GCT27 cells. Thus, it is conceivable that such a level of DNA repair cannot modulate significantly the cytotoxicity of DNA crosslinkers such as cisplatin in these cell lines and so any inhibition of DNA repair by F 11782 results in only minor effects in these GCT27 cells. Furthermore, potentiation of the therapeutic effects of DNA-damaging agents, such as cisplatin and mitomycin C by F 11782 was also shown in vivo using murine P388 leukaemia cells, in which significant DNA repair activity has been detected [23]. Indeed, the combination of suboptimal doses of F 11782 with either cisplatin or mitomycin C resulted in a higher level of antitumour activity (ILS) without any increased toxicity, relative to their individual effects as single treatments.

In conclusion, F 11782 appears to be a promising candidate for combining with other anticancer drugs,

since antagonism was rarely observed when it was combined simultaneously with each of nine antitumour agents. The inhibitory activity of F 11782 against NER provides a rationale to explain the effects of F 11782 in combination with DNA crosslinkers such as cisplatin or mitomycin C. However, any direct extrapolation of these experimental results to the clinic should be approached with great caution, since the definition of synergy is often quite different in in vitro studies, in in vivo experiments and in clinical evaluations. Nevertheless, several preclinical and clinical studies have already shown some benefits of combinations including topoisomerase I or II poisons and cisplatin without any clear mechanistic rationale [10, 33]. Thus, evaluations of F 11782 in combination with cisplatin or mitomycin C could be extended to other tumour types to provide further preclinical evidence of the potential of F 11782 in such combination chemotherapeutic approaches in the clinic.

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