Potentiation of Temozolomide and Topotecan Growth Inhibition and Cytotoxicity by Novel Poly(adenosine Diphosphoribose) Polymerase Inhibitors in a Panel of Human Tumor Cell Lines[1]

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ABSTRACT

Potent poly(ADP-ribose) polymerase (PARP) inhibitors have been developed that potentiate the cytotoxicity of ionizing radiation and anticancer drugs. The biological effects of two novel PARP inhibitors, NU1025 (8-hydroxy-2-methylquinazolin-4-[3*H*]one, K_i = 48 nM) and NU1085 [2-(4-hydroxyphenyl)benzamidazole-4-carboxamide, K_i = 6 nM], in combination with temozolomide (TM) or topotecan (TP) have been studied in 12 human tumor cell lines (lung, colon, ovary, and breast cancer). Cells were treated with increasing concentrations of TM or TP ± NU1025 (50, 200 μM) or NU1085 (10 μM) for 72 h. The potentiation of growth inhibition by NU1025 and NU1085 varied between the cell lines from 1.5- to 4-fold for TM and 1- to 5-fold for TP and was unaffected by p53 status. Clonogenic assays undertaken in two of the cell lines confirmed that the potentiation of growth inhibition reflected the potentiation of cytotoxicity. NU1025 (50 μM) was about as effective as 10 μM NU1085 at potentiating growth inhibition and cytotoxicity, consistent with the relative potencies of the two molecules as PARP inhibitors. Potentiation of cytotoxicity was obtained at concentrations of NU1025 and NU1085 that were not toxic *per se*; however, NU1085 alone was 3-fold more cytotoxic (LC_{50} values ranged from 83 to 94 μM) than NU1025 alone (LC_{50} > 900 μM). These data demonstrate that PARP inhibitors are effective resistance-modifying agents in human tumor cell lines and have provided a comprehensive assessment protocol for the selection of optimum combinations of anticancer drugs, PARP inhibitors, and cell lines for *in vivo* studies.

INTRODUCTION

The abundant nuclear enzyme PARP[3] (EC 2.4.2.30) is a 116-kDa enzyme that comprises an NH_2-terminal DNA-binding domain containing two zinc fingers that recognize DNA strand breaks, an automodification domain, and a COOH-terminal catalytic domain. PARP is activated by DNA strand breaks, and uses the ADP-ribose moiety of NAD^+ as substrate to synthesize long homopolymers of ADP-ribose on nuclear proteins. PARP itself is the main protein acceptor (automodification), but the enzyme has also been shown to modify histones, high mobility group proteins, topoisomerases, DNA polymerases, and ligases (reviewed in Refs. 1–3). The ADP-ribose polymers formed by PARP are degraded by poly(ADP-ribose) glycohydrolase (4), and after activation of PARP by DNA damage, the very rapid synthesis and degradation of ADP-ribose polymers that occurs can result in severe NAD^+ depletion (5).

PARP activation after DNA damage has pleiotropic functions, including mediation of DNA repair (*e.g.*, Refs. 6 and 7), modulation of p53 stability and function (8, 9), and regulation of apoptosis (reviewed in Ref. 10). Although precise molecular mechanisms for these functions have not been elucidated, the role of PARP in DNA BER has been well documented, using both PARP inhibitors and molecular genetic approaches. Recent evidence indicates that PARP is a member of a BER multiprotein complex, comprising PARP, DNA ligase III, *X*RCC, and DNA polymerase β, which is involved in the DNA synthesis step of BER (see Ref. 11 and references therein). PARP may also cooperate with DNA-dependent protein kinase in the regulation of DNA double strand break repair and in the maintenance of genomic stability by the prevention of unwanted recombination events (12–14). After DNA damage by alkylating agents, biochemical inhibition of PARP in cells mimics the altered responses in PARP knockout cells, namely, inhibition of DNA strand break repair and enhanced cytotoxicity (6, 7).

On the basis of its functional involvement in cell survival after DNA damage, PARP has been identified as a promising target for developing inhibitors for use in chemo- and radiopotentiation strategies, particularly because PARP function in the absence of extensive DNA damage is not essential for cell

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[3] The abbreviations used are: PARP, poly(ADP-ribose) polymerase; LC_{50}, concentration of drug causing 50% cytotoxicity; PF_{50}, potentiation factor at 50% growth inhibition; TM, temozolomide; TP, topotecan; NU1025, 8-hydroxy-2-methylquinazolin-4-[3*H*]one; NU1085, 2-(4-hydroxyphenyl)benzamidazole-4-carboxamide; BER, base excision repair.

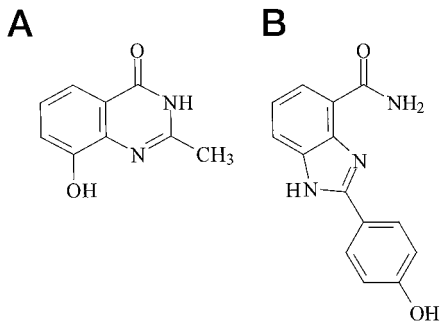

Fig. 1 Structures of NU1025 (K_i = 48 nM) (*A*) and NU1085 (K_i = 6 nM).

survival. This is exemplified by the survival and normal phenotype of knock out mice (15, 16). The potential of PARP inhibitors as resistance-modifying agents in cancer therapy has been comprehensively reviewed in Ref. (17). With rational drug design approaches, two structural classes of compounds have been identified as potent PARP inhibitors, namely, the benzimidazole-4-carboxamides and quinazolin-4-[3*H*]-ones (18–20). We have previously reported the ability of representatives of each of these classes, NU1025 (K_i = 48 nM) and NU1064 (K_i = 99 nM), to potentiate the cytotoxicity and inhibit the repair of DNA damage induced by DNA-methylating agents, ionizing radiation, and bleomycin in murine leukemia L1210 cells (6, 21).

The aim of the present study was to evaluate the growth-inhibitory and cytotoxic effects of novel PARP inhibitors used alone or in combination with clinically relevant anticancer drugs in a panel of human tumor cell lines. The 12 cell lines used represented 4 of the most common malignancies, namely, lung, breast, colon, and ovarian. They were selected on the basis of their reported p53 status to investigate whether or not cell lines harboring wild-type or mutant p53 showed differential susceptibility to PARP inhibitor-mediated potentiation. NU1025 and the 8-fold more potent benzamidazole NU1085 (K_i = 6 nM (19)), were selected as PARP inhibitors for this study (see Fig. 1 for structures). TM, a methylating agent showing promise in the treatment of melanomas and gliomas (22), was selected because the base methylation it induces promotes BER and because our previous studies have shown useful potentiation of cytotoxicity by NU1025 in L1210 cells (6). The selection of the topoisomerase I inhibitor TP, a camptothecin analogue, was based on observations that PARP inhibitors can potentiate camptothecin cytotoxicity (23–25). TP has shown a wide range of antitumor activity against adult and pediatric malignancies (26, 27).

The results presented here show that NU1025 and NU1085 potentiated both TM- and TP-induced growth inhibition and cytotoxicity in nearly all cell lines tested, irrespective of tumor origin or p53 status. NU1025 was cytostatic and cytotoxic in its own right, but the concentrations required to exert these effects were about an order of magnitude higher than those required to obtain potentiation when used in conjunction with TM or TP. In contrast, NU1085 displayed overlap between its inherent cytotoxic concentrations and those required for potentiation.

MATERIALS AND METHODS

Drugs. TM (a gift from the Cancer Research Campaign, London, United Kingdom) and TP (SmithKline Beecham Pharmaceuticals, Philadelphia, PA) were dissolved in DMSO at 10 and 2.2 mM, respectively, and stored as aliquots at −20°C. NU1025 and NU1085 were synthesized as previously described (18, 19). Stock solutions were prepared in DMSO at 100 mM and stored at −20°C. Drugs (alone or in combination) were added to cell cultures so that final DMSO concentrations were constant at 1% (v/v).

Cell Lines and Culture. A panel of human tumor cell lines representative of four common cancers were used: colon, HT29, LoVo, LS174T, breast: MCF-7, T47D, MDA-231; ovarian, SKOV-3, A2780, OAW-42; and lung A549, COR-L23, H522. The p53 status of the following cell lines has been characterized by DNA sequencing: A549 and MCF7, wild-type (28); LoVo, LST174T, and A2780, wild-type (29–31); H522, SKOV-3, HT29, MDA, and T47D, mutant (28); COR-L23, mutant (Dr. Xiaohong Lu, Cancer Research Unit, University of Newcastle upon Tyne, unpublished results). The p53 status of the OAW-42 cell line has not been reported to our knowledge. Cells were maintained as exponentially growing monolayers in RPMI 1640 supplemented with 10% (v/v) FCS (Sigma, Poole, United Kingdom), 1000 units/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Inc., Paisley, United Kingdom). In the case of the OAW-42 cell line, insulin (10 units/liter) was also added. Cells were tested every 4–8 weeks to exclude *Mycoplasma* contamination (32). The cell lines were obtained from either the European Collection of Animal Cell Cultures or the American Type Culture Collection, except for the COR-L23 cell line, a gift from Dr. P. Twentyman (United Kingdom Co-ordinating Committee for Cancer Research, London, United Kingdom).

Growth Inhibition Assays. Cells were plated at between 2.5×10^4 and 4×10^4/ml, dependent on cell line-doubling time, to ensure exponential growth during the course of the experiment, in 96-well plates (Nunc-Life A/S, Roskilde, Denmark), and incubated for 24 h. The medium was then replaced with medium containing TM or TP ± NU1025 or NU1085 (six replicates for each drug treatment). Controls containing either no drugs or NU1025 or NU1085 alone were also included. Replicate wells were fixed at this time to estimate cell number at the start of the drug incubation. After a 72-h exposure period cells were fixed, washed, and stained with sulforhodamine B as described previously (33). The absorbance of the wells relative to blank wells that contained no cells was measured on a computer-interfaced Dynatech MR7000 96-well microtiter plate reader (Dynatech, Billinghurst, United Kingdom) using a 570-nm filter. In single drug treatment experiments, drug-free controls containing 1% DMSO were included. In drug combination experiments, where a fixed concentration of PARP inhibitor was used in combination with increasing concentrations of TM or TP, the PARP inhibitor alone samples (*e.g.*, 50 μM NU1025) were used as controls. Similarly, when growth inhibition experiments were carried out with a fixed concentration of TM or TP in combination with increasing concentrations of PARP inhibitor, the controls for these experiments were the TM or TP alone samples. All control values were normalized to 100%. The values obtained for each of the six replicates were averaged, and IC_{50} values were

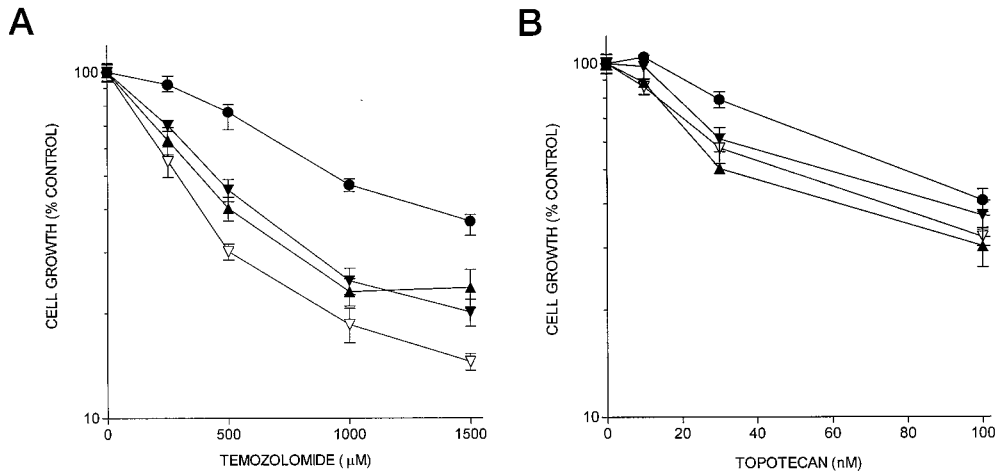

Fig. 2 Effects on growth of A549 cells of a 72-h exposure to increasing concentrations of TM ± fixed concentrations of NU1025 or NU1085 (*A*) and TP ± fixed concentrations of NU1025 or NU1085 (*B*). ●, control; ▼, + NU1025 (50 μM); ▽, + NU1025 (200 μM); ▲, + NU1085 (10 μM).

Table 1 Effect of NU1085 and NU1025 on TM-induced growth inhibition in human tumor cell lines

	IC_{50}[a] (μM)			
Cell line	TM	TM + 10 μM NU1085	TM + 50 μM NU1025	TM + 200 μM NU1025
A549 (lung)	949	394 (2.4)[b]	453 (2.1)	301 (3.2)
	1223	466 (2.6)	488 (2.5)	373 (3.3)
CORL23 (lung)	855	446 (1.9)	439 (1.9)	427 (2.0)
	984	681 (1.4)	977 (1.0)	719 (1.4)
H522 (lung)	>1500	1059 (>1.4)	837 (>1.8)	826 (>1.7)
	1249	705 (1.8)	524 (2.4)	645 (1.9)
SKOV3 (ovary)	>1500	639 (>2.3)	842 (>1.8)	427 (>3.5)
	>1500	867 (>1.7)	810 (>1.9)	339 (>4.4)
A2780 (ovary)	525	188 (2.8)	231 (2.3)	196 (2.7)
	368	183 (2.0)	203 (1.8)	188 (2.0)
OAW-42 (ovary)	568	419 (1.4)	485 (1.2)	364 (1.6)
	714	444 (1.6)	649 (1.1)	405 (1.8)
HT29 (colon)	>1500	881 (>1.7)	733 (>2.0)	415 (>3.6)
	>1500	719 (>2.1)	623 (>2.4)	429 (>3.5)
LoVo (colon)	>1500	828 (>1.8)	813 (>1.8)	474 (>3.2)
	>1500	821 (>1.8)	572 (>2.6)	277 (>5.4)
	>1500	929 (>1.6)	961 (>1.6)	611 (>2.5)
LS174T (colon)	>1500	432 (>3.5)	749 (>2.0)	421 (>3.6)
	1371	225 (6.1)	363 (3.8)	223 (6.1)
	>1500	766 (>2.0)	1133 (>1.3)	609 (>2.5)
MCF-7 (breast)	>1500	485 (>3.1)	461 (>3.3)	429 (>3.5)
	>1500	682 (>2.2)	990 (>1.5)	604 (>2.5)
T47D (breast)	>1500	747 (>2.0)	956 (>1.6)	834 (>1.8)
	>1500	1547	>1500	1150 (>1.3)
MDA-231 (breast)	>1500	1211 (>1.2)	1401 (>1.1)	786 (>1.9)
	1453	1152 (1.3)	1378 (1.1)	725 (2.0)

[a] IC_{50}s were calculated from data from individual experiments, using five replicates for all samples.

[b] Numbers in parentheses, PF_{50}s derived from the individual experiments. Where it was not possible to obtain IC_{50}s for TM alone because of the limited solubility of this drug (*i.e.*, IC_{50}s were not achieved by the highest concentration (1500 μM) of TM used), PF_{50}s were expressed as greater than the value calculated, assuming the IC_{50} for TM alone to be 1500 μM.

defined as the concentrations of drug(s) that inhibited growth by 50% relative to controls. The IC_{50} values were calculated from the growth inhibition curves generated by fitting sigmoidal curves to the data using unweighted nonlinear least square regression analysis (GraphPad Software, Inc., San Diego, CA). The PF_{50} was expressed as the ratio IC_{50} (control):IC_{50} (sample).

Clonogenic Survival Assays. Cell survival was determined by means of colony-forming assays. Cells were plated at

Table 2 Effect of NU1085 and NU1025 on TP-induced growth inhibition in human tumor cell lines

	IC_{50}[a] (nM)			
Cell line	TP	TP + 10 μM NU1085	TP + 50 μM NU1025	TP + 200 μM NU1025
A549 (lung)	>75	52 (>1.4)[b]	68 (>1.1)	40 (>1.9)
	70	19 (3.7)	32 (2.2)	17 (4.1)
	83	31 (2.7)	62 (1.3)	51 (1.6)
CORL23 (lung)	61	64 (0.9)	28 (2.2)	41 (1.5)
	61	64 (0.9)	28 (2.2)	25 (2.4)
H522 (lung)	>300	122 (>2.4)	81 (>3.7)	58 (>5.2)
	251	91 (2.8)	30 (8.4)	46 (5.5)
SKOV3 (ovary)	50	29 (1.7)	25 (2.0)	24 (2.1)
	23	19 (1.2)	16 (1.4)	12 (1.9)
	38	26 (1.5)	26 (1.5)	24 (1.6)
A2780 (ovary)	23	7.8 (2.9)	8.5 (2.7)	8.5 (2.7)
	22	7.9 (2.8)	8.7 (2.5)	8.1 (2.7)
OAW-42 (ovary)	25	21 (1.2)	20 (1.3)	12 (2.7)
	27	19 (1.4)	16 (1.7)	16 (1.7)
HT29 (colon)	66	23 (2.8)	45 (1.5)	38 (1.7)
	>75	45 (>1.7)	34 (>2.2)	37 (>2.0)
	72	28 (2.6)	28 (2.6)	34 (2.1)
LoVo (colon)	235	50 (4.7)	79 (3.0)	52 (4.5)
	291	92 (3.2)	90 (3.2)	71 (4.1)
LS174T (colon)	10	9 (1.2)	8 (1.3)	8 (1.3)
	10	10 (0.9)	8 (1.3)	9 (1.2)
MCF-7 (breast)	>300	95 (>3.2)	168 (>1.8)	98 (>3.1)
	>300	155 (>1.9)	233 (>1.3)	149 (>2.0)
T47D (breast)	92	29 (3.2)	24 (3.8)	34 (2.7)
	271	229 (1.2)	211 (1.3)	210 (1.3)
MDA-231 (breast)	>300	>300	>300	>300
	>300	>300	136 (>2.2)	>300

[a] Data were from individual experiments, using five replicates for all samples.
[b] Numbers in parentheses, PF_{50} values derived from the individual experiments. Where IC_{50}s were not attained by the highest concentration of TP used (300 nM), PF_{50}s were expressed as greater than the value calculated, assuming the IC_{50} for TP alone to be 300 nM.

a density of 2×10^4 cells/ml for 24–48 h before treatment. All cell lines were treated with TM or TP ± NU1025 or NU1085 for 24 h. After the exposure period, the cells were trypsinized, resuspended in medium, and counted with a Coulter Counter (model Z1, Coulter Electronics, Bedfordshire, United Kingdom). A known number of cells were seeded onto 10-cm plastic Petri dishes to allow colony formation. After 2 weeks, colonies were fixed and stained with crystal violet (*N*-hexamethylpararosaniline). Survival was calculated as a percentage of control for each drug concentration (see definition of "control" in previous section).

RESULTS

Potentiation of TM and TP Growth Inhibition by NU1025 and NU1085. The abilities of NU1025 and NU1085 to potentiate the growth-inhibitory effects of TM and TP were evaluated in all 12 cell lines. Using the K_i values as a guide, (48 and 6 nM, respectively, for NU1025 and NU1085), concentrations of NU1025 (50 μM) and NU1085 (10 μM) were selected to achieve approximately similar levels of PARP inhibition in cell culture. In addition, a higher concentration of NU1025 (200 μM) was used, one previously demonstrated to produce maximal potentiation of TM cytotoxicity in L1210 murine leukemia cells (6). These concentrations of inhibitors *per se* were slightly growth inhibitory (≤20%); this was accounted for in the analyses of the results (see "Materials and Methods"). The effects of these fixed concentrations of NU1025 and NU1085 on growth inhibition produced by continuous exposure to increasing concentrations of TM or TP during a 72-h incubation were investigated in all 12 cell lines. Representative growth inhibition curves for the A549 cell line are shown in Fig. 2, where it can be seen that 10 μM NU1085 potentiated both TM and TP growth inhibition at least 2-fold, and to about the same extent as 50 μM NU1025, with 200 μM NU1025 producing greater potentiation.

IC_{50} values for TM or TP ± NU1025 (50, 200 μM) or NU1085 (10 μM) were computed, and PF_{50} values were derived. Sensitivity to TM alone (Table 1) ranged from IC_{50} 447 μM in the A2780 cell line to IC_{50} >1500 μM for eight of the other cell lines. (IC_{50} values >1500 μM could not be accurately determined due to the limited solubility of TM in DMSO). For the cell lines where PF_{50} values could be calculated for TM + NU1025 or NU1085, these values ranged from >5 to little greater than 1 (*i.e.*, almost no potentiation by the inhibitors). A 200 μM concentration of NU1025 consistently gave higher PF_{50} values than 50 μM NU1025, suggesting that complete cellular inhibition of PARP had not been achieved at the lower concentration of the inhibitor. No obvious tissue specificity for high *versus* low PF_{50} values was observed.

Similar data for TP-induced growth inhibition are summarized in Table 2. The range of sensitivities to TP alone in the cell

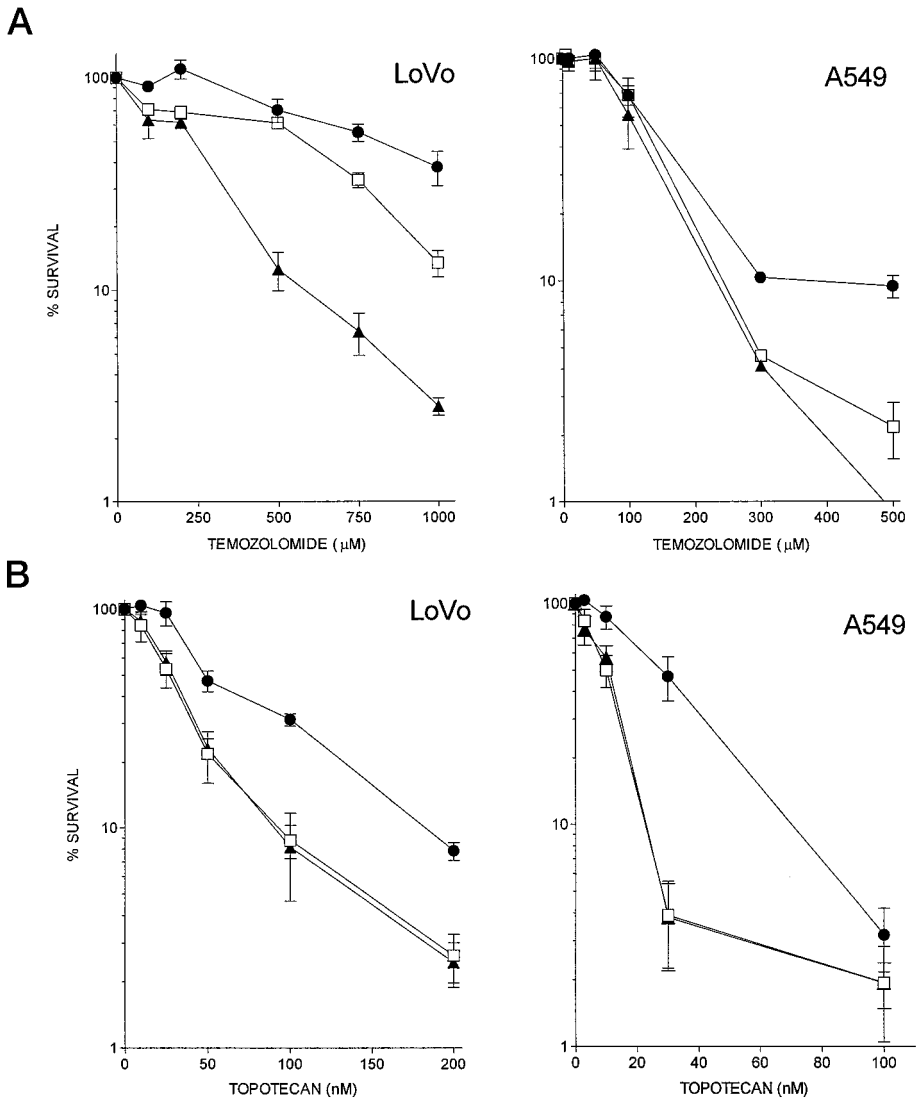

Fig. 3 Effect of a 24-h exposure of cells to increasing concentrations of TP or TM, in the presence or absence of NU1025 (200 μM) or NU1085 (10 μM), on clonogenic survival. ●, control; ▲, NU1025; □, NU1085.

lines was much broader than with TM, with IC_{50} values ranging from >300 nM in three of the cell lines (MCF7, MDA-231, and H522) to 10 nM (LS147T). With the exception of the latter cell line, the three ovarian cell lines were notably more sensitive to TP than all of the other tumor types. The majority of the PF_{50} values were between 2 and 4, although the cell line most sensitive to TP alone (LS147T) displayed little or no potentiation of growth inhibition by the PARP inhibitors (values from 0.9 to 1.3). For four of the cell lines (A549, SKOV, LoVo, and MCF7), 200 μM NU1025 gave higher PF_{50} values than 50 μM. For the remaining cell lines, there was no persuasive increase in the PF_{50} values at 200 μM compared with 50 μM, and indeed, in some cases, the PF_{50} values at 50 μM were higher than at 200 μM. However, because of the high number of cell lines involved, the majority of the data represent two independent experiments only and were not amenable to statistical analyses.

As predicted from their relative potencies as inhibitors of purified PARP, 10 μM NU1085 was about as effective as 50 μM NU1025, and this equivalence was maintained with both TM and TP in all of the cell lines (see Tables 1 and 2).

Potentiation of TM and TP Clonogenic Cytotoxicity by NU1025 and NU1085. Growth inhibition does not necessarily result in cytotoxicity; some drugs exert reversible cytostatic effects with minimal effects on cell survival. Clonogenic survival assays were therefore performed to ascertain whether the enhanced growth-inhibitory effects produced by NU1025 and NU1085 correlated with increased cell killing.

Three of the twelve cell lines, LoVo, A549, and OAW-42, were selected for all subsequent studies, and survival curves for A549 and LoVo are shown in Fig. 3. TM proved to be considerably more cytotoxic than cytostatic. (The half-life of TM is <2 h; thus, the shorter exposure time in this experiment, 24 h compared with 72 h, is not relevant (34)). For example, ~200 μM TM reduced clonogenic survival by 50% in the LoVo cell line, whereas the IC_{50} value for growth inhibition was >1500 μM (compare Fig. 3 and Table 1), and the same trend was

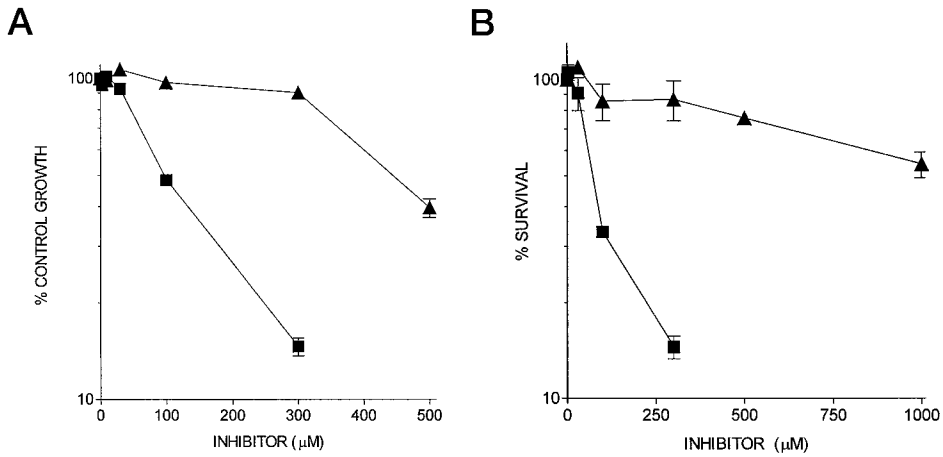

Fig. 4 Comparison of the growth-inhibitory and cytotoxic effects of exposure to NU1025 or NU1085 in LoVo cells. *A,* effect on growth of a 72-h continuous exposure to NU1025 ▲ or NU1085 ■; *B,* effect on survival of a 24-h exposure to NU1025 ▲ or NU1085 ■.

Table 3 Comparison of the growth-inhibitory and cytotoxic effects of NU1025 and NU1085 as single agents[a]

Cell line	NU1025 IC_{50} (μM)	NU1025 LC_{50} (μM)	NU1085 IC_{50} (μM)	NU1085 LC_{50} (μM)
A549	>1000	>1000, >1000, >1000	83 ± 18	74 ± 8
LoVo	330 ± 139	901, >1000, >1000	94 ± 11	70 ± 24
OAW-42	263 ± 119	920 ± 67	82 ± 8	80 ± 20

[a] Data are presented either as the mean of three or more independent experiments ± SD or as the results of individual experiments. IC_{50}s were calculated as described in "Materials and Methods." LC_{50}s were determined directly from the survival curves, which were plotted point to point.

observed with the A549 cells. NU1025 and NU1085 (at 200 and 10 μM, respectively) potentiated the cytotoxicity of TM in both cell lines, in general agreement with the results obtained for growth inhibition in Table 1. TP cytotoxicity was also potentiated to similar extents by coincubation with NU1025 and NU1085, and again these results were consistent with the potentiation of growth inhibition observed.

Cytostatic and Cytotoxic Effects of NU1025 and NU1085 Alone. As has been previously stated, both PARP inhibitors were used in the growth inhibition and clonogenic survival assays at concentrations which by themselves showed modest (≤20%) growth inhibition. The concentration-dependent effects of NU1025 and NU1085 on growth and survival in the absence of TM and TP were assessed in more detail. Fig. 4 shows representative growth inhibition and survival experiments for LoVo cells exposed either continuously (72 h) to increasing concentrations of NU1025 or NU1085 for growth inhibition analysis (Fig. 4*A*) or for 24 h before plating for survival in the absence of inhibitor (Fig. 4*B*). NU1085 was considerably more growth inhibitory and cytotoxic than NU1025.

A summary of the IC_{50} and LC_{50} values for both compounds is given in Table 3 for three of the cell lines. Notably, whereas NU1025 was about 3-fold more cytostatic than cytotoxic in the OAW-42 and LoVo cell lines, the cytostatic and cytotoxic potencies of NU1085 were nearly equivalent. The IC_{50} values for NU1085 clustered between 80 and 100 μM, and were in close agreement with the LC_{50} values. In comparison, the IC_{50} values for NU1025 were ≤330 μM for two of three cell lines, whereas the LC_{50} values were ≥920 μM for all three.

Separation of the Potentiating and Direct Cytotoxic Effects of NU1025 and NU1085. A DNA repair inhibitor for use in chemotherapy should ideally exert no toxic effects *per se* in the absence of DNA damage. As can be seen from the above results, NU1085 was >10-fold more cytotoxic than NU1025. With NU1025, the LC_{50} values when used alone (=900 μM) were about an order of magnitude higher than the concentrations of the inhibitor (50–200 μM) required to potentiate TM and TP (compare Tables 1, 2, and 3). An experiment was designed to compare quantitatively the concentration-dependent effects of NU1025 on these two biological end points. LoVo cells were exposed to increasing concentrations of NU1025 in the presence or absence of a single fixed concentration of TM (1 mM), which itself caused ~20% growth inhibition, and growth inhibition and clonogenic survival determined respectively. A comparison of the data for growth inhibition (Fig. 5*A*) and clonogenic survival (Fig. 5*B*) confirms that potentiation of TM could be achieved at concentrations of NU1025 that exerted no growth-inhibitory or cytotoxic effects when used by itself. Furthermore, a concentration-dependent increase in the extent of potentiation of TM-induced growth inhibition and cytotoxicity by NU1025 was obtained. Maximal potentiation was achieved by ~300 μM NU1025 in the growth inhibition experiments and 500 μM NU1025 in the clonogenic survival experiments. This concentration-dependent increase in the extent of the potentiation of TM was found in all of the cell lines tested, by both NU1025 and NU1085, and is consistent with the results in Table 1 for potentiation of TM-induced growth inhibition, where the PF_{50} values for 200 μM NU1025 were consistently higher than the 50 μM values. However, in contrast to NU1025, NU1085 alone

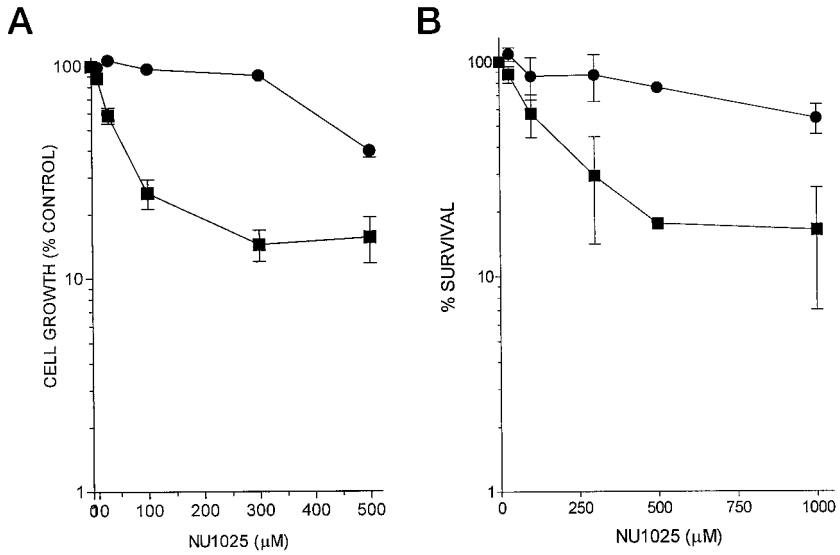

Fig. 5 Effects on growth (*A*) and clonogenic survival (*B*) of LoVo cells treated with increasing concentrations of NU1025 alone (●) or in the presence of a fixed concentration (1 mM) of TM (■).

became cytotoxic at concentrations at which its potentiating effects were still increasing (results not shown).

DISCUSSION

PARP inhibitors have been available for nearly two decades, and their clinical potential as adjuvants to anticancer therapies has long been recognized (reviewed in Ref. 17). More recently, considerable interest has also focused on their use in preventing the toxic effects of inflammatory damage after transient ischemia (10). However, it is only with the development of high potency inhibitors that their clinical application has become a realistic prospect. It is timely, therefore, that the efficacy of currently available inhibitors should be subject to systematic assessment in human tumor cell lines with clinically used anticancer drugs, as has been described here.

An evaluation of 12 human tumor cell lines was conducted with the intention of establishing whether there were marked differential sensitivities or selectivities to the PARP inhibitors, either in their ability to potentiate TM or TP, or in their inherent toxicities *per se*. The comparison of growth inhibition by the sulforhodamine B assay with clonogenic survival demonstrated that the far more rapid sulforhodamine B screen produced results consistent with the survival data and validated the former technique as a sufficient single method for studies with multiple cell lines. Although the PF_{50} values for the two inhibitors varied between cell lines, there was no single tissue type that displayed unusually marked or limited potentiation with either TM or TP. Furthermore, although both p53 mutant and WT cells were represented in the panel of cell lines (see "Materials and Methods" for details), no differential sensitivity to potentiation by the PARP inhibitors was noted. These observations suggest that PARP inhibitors as chemotherapeutic tools will not be limited by either cancer type or p53 status.

Although the K_i values for NU1025 and NU1085 were 48 and 6 nM, respectively, micromolar concentrations were required in cell culture to produce significant potentiation. In general, 10 μM NU1085 was about as effective as 50 μM NU1025 in the growth inhibition assays, with 200 μM NU1025 showing greater potentiation in the majority of experiments, indicating that PARP inhibition was not maximal at the lower concentrations. This was confirmed by assessing the concentration-dependent effects of NU1025 on TM-induced growth inhibition, where maximal potentiation was not achieved until ~300 μM NU1025 (see below). The extent of PARP inhibition achieved in cell culture will depend on factors such as inhibitor stability, membrane diffusion, and/or transport, intracellular distribution, and metabolic inactivation, as well as PARP and NAD^+ levels in the cell lines.

A method for quantitatively assessing the relative potency of the inhibitors as potentiators of cytotoxicity was devised by inverting the conventional protocol of assessing the growth-inhibitory or cytotoxic effects of increasing concentrations of an anticancer agent in the presence or absence of a fixed concentration of resistance modifier (in this case, a PARP inhibitor). Thus, cells were treated with increasing concentrations of NU1025 in the presence or absence of a fixed concentration of TM, which itself caused only limited toxicity. This methodology proved useful for determining the optimum concentration of PARP inhibitor for maximal potentiation in cell culture, and also the ratio of the PARP inhibitor concentration required for potentiation to that which produced growth inhibition/cytotoxicity in its own right. Resistance modifiers, such as PARP inhibitors, should ideally be active at doses or concentrations that are nontoxic, and in this study NU1025 clearly fulfilled this criterion.

The data presented herein provide a comprehensive preclinical *in vitro* evaluation of the potential therapeutic efficacy and potency of chemotherapeutic agent-PARP inhibitor combinations. The development of this screen has facilitated the selection of the most suitable PARP inhibitors for studies with human tumor xenografts in nude mice and, ultimately, for clinical trials.

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