Studies of synergistic and antagonistic combinations of conventional cytotoxic agents with the multiple eicosanoid pathway modulator LY 293111

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The arachidonic acid metabolic pathway is currently under active investigation as a promoter of malignancy and several molecules have been synthesized to block either the cyclooxygenase or lipoxygenase branches. LY 293111 is an oral agent known to be a leukotriene B4 antagonist, a 5-lipoxygenase inhibitor and a peroxisome proliferator-activated receptor (PPAR)-γ agonist with cytotoxic properties in cell lines. We have studied this agent with classical chemotherapeutic agents in a 72-h culture with cell lines using median-effect analysis as a measure of antagonism or synergy. LY 293111 displays global synergy with the active metabolite of irinotecan, SN-38, in the majority of cell lines, synergistic to additive effects with gemcitabine in bladder cancer cell lines, and synergism with 5′-DFUR (the active metabolite of capecitabine) in two breast cancer and one sarcoma cell line. These effects occur at clinically attainable concentrations. The addition of a proteosome inhibitor to the LY 293111 and SN-38 combination markedly enhanced the cytotoxic effects in the sarcoma cell line. As the toxicity of LY 293111 in man is not hematological, this agent may have a role in combination therapy of selected malignancies.

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Introduction

Most of the present chemotherapeutic combinations used for the treatment of human solid tumors have been developed on an empiric basis by adding active agents together. However, this approach may result in drug interactions in which the combination may demonstrate additive, synergistic or antagonistic cytotoxic effects *in vitro*, thus questioning the validity of at least some combination therapies in the clinical setting [1]. In an attempt to make the selection of combination chemotherapy more rational, we and others have used the technique of median-effect analysis to examine, in preclinical models, the effects of combining active agents together [1–7]. The advantage of median-effect analysis is that it requires less experimental determinations than classical isobologram analysis, can be semiautomated and can evaluate up to three different agents at a time [6]. Clinically active combinations such as estramustine/docetaxel, vinorelbine/taxanes and trastuzumab with vinorelbine have been identified by this technique [4,8–10]. We have also used this technique to identify synergistic combinations of targeted agents with classical cytotoxic drugs [11].

The leukotriene B4 (LTB4) is an important mediator in the 5-LO/LTA4 hydrolase pathway [12]. This leukotriene has been demonstrated to enhance the growth of colon cancer cell lines (HT-29 and HCT-15) [13], to possibly be involved in growth control for the breast cancer cell line MDA-MB-231 [14] and is generated from precursors by melanoma cells [15]. Recent investigations of this pathway and the receptor antagonist in six human pancreatic cell lines has demonstrated that LTB4 has a growth stimulatory role, and inhibition of this agonist leads to apoptosis of the pancreatic carcinoma cells in a time- and concentration-dependent manner [16].

LY 293111 is an oral agent known to be a LTB-4 antagonist, 5-lipoxygenase inhibitor and a peroxisome proliferator-activated receptor (PPAR)-γ agonist [17]. LY 293111 also demonstrated marked anti-inflammatory effects in a murine model [18]. The agent is orally available with reproducible pharmacodynamic effects in man [19]. As a consequence of the above biological effects, LY 29311 has undergone a formal phase I evaluation in cancer patients with dose linear pharmacology and major dose-limiting effects of diarrhea [20]. The agent is currently in phase II trials.

As this agent has a unique mode of action which is different from classical chemotherapeutic agents and since common malignancies may be influenced by

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leukotriene B4 acting as an agonist, we have examined LY 293111 in combination with more traditional therapy and herein report our findings.

Material and methods

Nine cell lines (MCF_7, BT 474, SK-BR-3, LL 86, H460, SW 480, COLO320/HSR, RT 4 and HT 1197) were obtained from the ATCC (Rockville, MD) with one multiply resistant breast cancer cell line, MCF_7/ADR obtained from the National Cancer Institute (NCI, Bethesda, MD). The cell lines were stored at −80°C prior to culture to limit the number of passages and then were grown in log phase for the experiments. LY 293111 (reagent grade) was a gift of the Eli Lilly (Indianapolis, IN), SN-38 (the active metabolite of irinotecan) a gift of Pfizer Pharmaceuticals (New York, NY), docetaxel (reagent grade) obtained from Aventis Pharmaceuticals (Parsippany, NJ), trastuzumab from commercial stock and all other reagents from Sigma-Aldrich (St Louis, MO).

The experimental conditions were previously reported in detail [1,4,8]. In brief, cells were grown to confluence in T 150 tissue culture flasks (Corning Glass Works, Corning, NY) using RPMI 1640 (Gibco, Grand Island, NY) with 5% CO_2 and 10% fetal bovine serum, McCoy's 5A medium, or Minimal Essential Medium depending upon ATCC directions. All cultures contained penicillin (100 μg/ml) streptomycin (0.25 μg/ml) and glutamine to a final concentration of 2 mM. Cells were harvested, washed, and aliquoted in 96-well dishes (Falcon 3072; Baxter Scientific, McGraw Park, IL) at concentrations of 5000–8000 cells/well in a total volume of 200 μl/well. All cells had viability by Trypan blue exclusion over 95% and were negative for mycoplasma.

The cells were allowed to adhere to the microtiter plate for 24 h and then exposed to culture media containing either vehicle or drug for 72 h. Cell growth was determined by a MTT assay using a Bio-Rad 3550 Microplate Reader (Bio-Rad, Hercules, CA). IC_{50} (the dose of drug needed to cause cytotoxicity in 50% of the cells) concentrations were determined singly for each drug over a 72-h incubation period by the EZ-ED50 Program (Perrella Scientific, Conyers, CA). All reported values are the means of at least three experiments with each study having four wells per dose level.

Median-effect analysis, based upon the Hill equation, allowed the determination of synergistic, additive or antagonistic effects when up to three agents were combined together. This effect was determined by the method of Chou [6,7] using their computer program [21]. The resulting combination index (CI) which reflects synergy when less than 1, additive effects when equal to 1 and antagonism when greater than 1 was calculated for varying levels of drug effect (F_a). We have defined additive effects to be all values within 1 SD of unity. Ten fixed drug ratios above and below the IC_{50} with a range of $0.0156N$ to $8N$, where N is a value near the IC_{50} of an individual drug, were explored by incubating the drug combinations with cells for 72 h and then determining the degree of cytotoxic effect by the MTT assay. F_a is defined as the fraction of cells affected and a plot of log dose versus log $F_a/(1-F_a)$ gives parallel slopes if no biologic interaction is present (mutually exclusive) or converge if there is an interaction between the drugs (mutually non-exclusive), thus suggesting the appropriate model to determine the CI [21]. F_{a50} is defined at that point where 50% of the cells are affected (killed as defined by the MTT assay). The results of the drug interactions are shown in tabular form at the F_{a50} as the median effect equation is a linear approximation of a higher-order equation and most accurate at the F_{a50}.

Results

Table 1 displays the single-agent cytotoxic effect of the various drugs in these cell lines using a 72-h incubation time. As expected, the IC_{50}s vary widely with the agents tested and the cell lines used. SN-38 was the most cytotoxic single agent tested with the bladder cell lines demonstrating an order of magnitude more sensitivity.

Median-effect analysis has been explored in the 10 cell lines as shown in Table 2. Plots of log dose versus log $F_a/(1-F_a)$ converged, suggesting the use of the mutually non-exclusive criterion for determination of CI. The drug combinations used reflect the known clinical utility of these drugs in the given tumor type. In addition, the use

Table 1 **IC_{50} of the individual drugs incubated with cells for 72 h**

Drug	Cell line/tumor type							
	MCF7/wt (breast)	MCF7/adr$^+$ (breast)	LL 86 (lung)	H 460 (lung)	SW 480 (colon)	COLO/HSR (colon)	RT 4 (bladder)	HT 1197 (bladder)
5′-DFUR	6.02 ± 0.62	6.58 ± 2.46	21.63 ± 3.02	2.89 ± 0.50	12.72 ± 1.25	6.07 ± 0.94	24.57 ± 6.71	21.31 ± 3.37
Cisplatin	5.32 ± 0.51	2.76 ± 0.18	6.12 ± 1.85	8.77 ± 0.45	37.38 ± 11.23	122.94 ± 12.78	4.40 ± 1.32	3.64 ± 0.93
Gemcitabine	2.06 ± 0.41	2.13 ± 0.84	1.54 ± 0.70	2.58 ± 0.55	2.89 ± 1.45	0.038 ± 0.013	0.094 ± 0.043	4.51 ± 2.03
LY 293111	9.36 ± 0.93	30.88 ± 4.56	8.58 ± 1.45	26.04 ± 1.95	33.93 ± 3.84	8.16 ± 1.12	26.72 ± 4.44	7.88 ± 1.88
MG 132	0.46 ± 0.08	3.86 ± 0.37	15.99 ± 4.29	0.45 ± 0.18	0.24 ± 0.04	0.90 ± 0.19	0.080 ± 0.019	0.44 ± 0.15
SN-38	0.54 ± 0.32	0.19 ± 0.03	0.30 ± 0.05	0.04 ± 0.02	0.06 ± 0.02	0.23 ± 0.02	0.003 ± 0.001	0.006 ± 0.001

All values are given in μM and are means ± SD of three to six experiments.

Table 2 Median-effect analysis of doublet and triplet combinations of agents with potential clinical utility

Drug combination	Cell line/tumor type									
	MCF7/wt (breast)	MCF7/adr$^+$ (breast)	BT 474 (breast)	SK-BR-3 (breast)	LL 86 (lung sarcoma)	H 460 (lung)	SW 480 (colon)	COLO/HSR	RT 4 (colon)	HT 1197 (bladder)
Doublets										
LY 293111 + cisplatin	1.36 ± 0.08	2.97 ± 0.45	1.05 ± 0.03	1.05 ± .05	1.46 ± 0.03	1.14 ± 0.11	1.21 ± 0.07	0.31 ± 0.02	0.12 ± 0.01	3.89 ± 0.45
LY 293111 + 5′-DFUR	2.77 ± 0.47	3.48 ± 0.86	0.94 ± 0.09	0.51 ± 0.18	0.58 ± 0.05	1.28 ± 0.02	1.74 ± 0.27	2.53 ± 0.21	2.78 ± 0.25	0.60 ± 0.11
LY 293111 + gemcitabine	2.82 ± 0.37	1.47 ± 0.27	1.43 ± 0.09	2.17 ± 0.18	5.84 ± 0.64	1.62 ± 0.17	0.23 ± 0.02	2.31 ± 0.28	1.11 ± 0.12	0.40 ± 0.10
LY 293111 + MG 132	0.26 ± 0.06	1.15 ± 0.06	1.20 ± 0.09	1.56 ± 0.09	1.13 ± 0.09	3.11 ± 0.77	0.27 ± 0.03	2.27 ± 0.12	2.81 ± 0.20	1.12 ± 0.22
LY 293111 + SN-38	0.86 ± 0.08	0.70 ± 0.05	1.34 ± 0.09	0.96 ± 0.16	6.98 ± 0.62	0.64 ± 0.01	0.49 ± 0.06	0.79 ± 0.12	0.85 ± 0.02	0.74 ± 0.06
Triple combinations										
LY 293111 + SN-38 + MG 132	1.41 ± 0.13	0.85 ± 0.01	0.59 ± 0.05	1.16 ± 0.03	0.51 ± 0.07	0.57 ± 0.05	1.31 ± 0.15	0.30 ± 0.02	0.15 ± 0.01	3.28 ± 0.46
LY 293111 + cisplatin + gemcitabine	0.99 ± 0.11	0.98 ± 0.26	1.65 ± 0.33	1.06 ± 0.12	0.58 ± 0.08	0.85 ± 0.07	0.48 ± 0.05	0.57 ± 0.07	1.76 ± 0.42	0.66 ± 0.10

All values are the combination index at F_{a50} as means ± SD of two to four experiments.

of LY 293111 with cell lines of the various tumor types was chosen to reflect ongoing clinical studies and/or the interest of the investigators. Both doublet and triplet combinations of drugs were studied. A global effect was noted with the combination of SN-38 and LY 293111 which demonstrated cytotoxic synergy when applied to six of the eight cell lines studied. A plot of CI versus F_a is shown for this combination in the colon line SW 480 (Fig. 1). One sarcoma cell line, LL86, demonstrated antagonism for this two-drug combination and one breast cancer cell line, SK-BR-3, demonstrated additivity. Gemcitabine demonstrated additive to synergistic effects in the bladder cell lines when combined with LY 293111 (Table 2). The clinically useful combination of gemcitabine/cisplatin combined with LY 293111 also demonstrated strong synergistic effects (CI = 0.66) in the bladder cancer cell line HT 1197. This triplet was also tested in the breast cancer cell lines giving additive effects in three out of the four lines. LY 293111 was additive with 5′-DFUR in BT 474 (estrogen receptor-positive) and synergistic with 5′-DFUR in SK-BR-3 (estrogen receptor-negative), two cell lines representing aggressive breast cancer. This doublet also demonstrated synergistic effects in the sarcoma cell line LL 86, a particularly resistant cell line.

Fig. 1

Median-effect plot of the entire cytotoxic effect (F_a) for the combination of SN-38 and LY 293111 in colon cancer cell line SW 480.

As proteosome inhibition is a new targeted therapy undergoing extensive clinical trials [22], the proteosome inhibitor MG 132 was combined with LY 293111, giving synergistic effects in MCF7/wt and antagonistic effects in MCF7/adr and SK-BR-3 breast cancer cell lines. Cell line specific effects were seen in the other assays (Table 2). The triplet of MG 132, LY 293111 and SN-38 also demonstrated synergy in four out of the eight cell lines tested. Potentially of interest, the addition of a proteosome inhibitor to LY 293111 and SN-38 resulted in marked cytotoxic synergism (CI = 0.5) in the sarcoma cell line LL 86 in contrast to the doublet of SN-38 and LY 293111 which caused antagonism of cytotoxic effect (Table 2).

Discussion

With the advent of recent advances in genomics and bioinformatics, new targets with potential therapeutic applications are rapidly being developed leading to non-traditional anti-cancer agents. Many of these target directed agents act upon cell surface receptors and/or signal transduction pathways [23–27]. The arachidonic acid pathway, which involves both cyclooxygenase and lipoxygenase, is an active area of investigation as a potential target both in chemoprevention of malignancy and as a target for established malignancy [28–31]. Recent early clinical data has also suggested that the combination of a cyclooxygenase inhibitor with classical chemotherapy may have a role in lung cancer treatment [32]. LY 293111, an oral agent known to be a LTB4 antagonist, a 5-lipoxygenase inhibitor and a PPAR-γ agonist, is therefore potentially interesting as an agent to be used in combination with established agents. As PPAR-γ agonists suppress transcriptional activation of cyclooxygenase-2 [33] and also block AP-1 and NF-κB-mediated functions [34], but in a cell line-specific fashion [33,35], this agent may act through a variety of mechanisms.

Using our *in vitro* screening technique, we have identified the combination of LY 293111 and SN-38, the active metabolite of irinotecan, as being an attractive combination for further studies as the combination is synergistic in a variety of cancer cell lines suggesting a global class effect. As topoisomerase I inhibitors are assuming a greater role in clinical cancer therapy, LY 293111 needs to be evaluated with such agents. The dosages studied are clinically obtainable. Gemcitabine was also active with LY 293111 in bladder cancer cell lines, thus suggesting a potential role in this disease. In addition, the active metabolite of capecitabine, 5′-DFUR, was active in two of the four breast cancer cell lines, suggesting a potential use in more aggressive disease. 5′-DFUR with LY 293111 was also active in the sarcoma cell line LL 86.

The difficulty of extrapolating from this *in vitro* system is that it involves homogenous cell lines, uses exponential phase cell growth in a two dimensions (not a spheroid) and cannot identify therapeutic index (toxicity to the tumor compared to the host). However, with these caveats, we have been able to identify several potentially interesting combinations which may be advanced to further preclinical and potentially clinical studies. This methodology also allowed us to identify antagonistic combinations which should not be developed further.

References

1 Budman DR, Calabro A, Kreis W. *In vitro* evaluation of synergism or antagonism with combinations of new cytotoxic agents. *Anticancer Drugs* 1998; **9**:697–702.

2 Adjei AA, Budihardjo II, Rowinsky EK, Kottke TJ, Svingen PA, Buckwalter CA, *et al.* Cytotoxic synergy between pyrazoloacridine (NSC 366140) and cisplatin *in vitro*:inhibition of platinum–DNA adduct removal. *Clin Cancer Res* 1997; **3**:761–770.

3 Kahan BD, Gibbons S, Tejpal N, Chou TC, Stepkowski S. Synergistic effect of the rapamycin–cyclosporine combination: median effect analysis of *in vitro* immune performances by human T lymphocytes in PHA, CD3, and MLR proliferative and cytotoxicity assays. *Transplant Proc* 1991; **23**:1090–1091.

4 Budman DR, Calabro A, Wang LG, Liu XM, Stiel L, Adams LM, *et al.* Synergism of cytotoxic effects of vinorelbine and paclitaxel *in vitro*. *Cancer Invest* 2000; **18**:695–701

5 Budman DR, Calabro A, Kreis W. *In vitro* effects of dexrazoxane (Zinecard) and classical acute leukemia therapy: time to consider expanded clinical trials? *Leukemia* 2001; **15**:1517–1520.

6 Chou TC, Tan QH, Sirotnak FM. Quantitation of the synergistic interaction of edatrexate and cisplatin *in vitro*. *Cancer Chemother Pharmacol* 1993; **31**:259–264

7 Chou TC, Motzer RJ, Tong Y, Bosl GJ. Computerized quantitation of synergism and antagonism of taxol, topotecan, and cisplatin against human teratocarcinoma cell growth: a rational approach to clinical protocol design. *J Natl Cancer Inst* 1994; **86**:1517–1524.

8 Kreis W, Budman DR, Calabro A. Synergistic and antagonistic combinations of drugs in human prostate cancer cell lines *in vitro*. *Br J Urol* 1997; **79**:196–202

9 Budman DR, Calabro A, Kreis W. *In vitro* search for synergy and antagonism: evaluation of docetaxel combinations in breast cancer cell lines. *Breast Cancer Res Treat* 2002; **74**:41–46

10 Burstein HJ, Kuter I, Campos SM, Gelman RS, Tribou L, Parker LM, *et al.* Clinical activity of trastuzumab and vinorelbine in women with HER2-overexpressing metastatic breast cancer. *J Clin Oncol* 2001; **19**: 2722–2730

11 Budman DR. New directions with capecitabine combinations in advanced breast cancer. *Oncology* 2002; **16**:23–28

12 Yokomizo T, Izumi T, Shimizu T. Leukotriene B4: metabolism and signal transduction. *Arch Biochem Biophys* 2001; **385**:231–214

13 Cristiana B, Hanif R, Kashfi K. The effect of leukotriene B4 and selected HETEs on the proliferation of colon cancer cells. *Biochim Biophys Acta* 1996; **1300**:240–246

14 Earashi M, Noguchi M, Tanaka M. *In vitro* effects of eicosaniod synthesis inhibitors in the presence of linoleic acid on MDA-MB-231 human breast cancer cells. *Breast Cancer Res Treat* 1996; **37**:29–37

15 Okano M, Ikai K, Imamura S. Human melanoma cells generate leukotriene B4 and C4 from leukotriene A4. *Arch Dermatol Res* 1997; **289**:347–351

16 Tong WG, Ding XZ, Hennig R, Witt RC, Standop J, Pour PM, *et al.* Leukotriene B4 receptor antagonist LY 293111 inhibits proliferation and induces apoptosis in human pancreatic cancer cells. *Clin Cancer Res* 2002; **8**:3232–3242

17 Perkins E, Cramer J, Farid N, Gadberry M, Jackson D, Mattiuz E, *et al.* Preclinical characterization of 2-[3-[3-[5-ethyl-4′-fluoro-2-hydroxy[1,1′-biphenyl]-4-yl]oxy]propoxy]-2-propylphenoxy]benzoic acid metabolism: *in vitro* species comparison and *in vivo* disposition in rats. *Drug Metab Disp* 2003; **31**:1382–1390

18 Kuwabara K, Yasui K, Jyoyama H, Maruyama T, Fleisch JH, Hori Y. Effects of the second-generation leukotriene B(4) receptor antagonist, LY 293111Na, on leukocyte infiltration and collagen-induced arthritis in mice. *Eur J Pharmacol* 2000; **402**:275–285

19 Marder P, Sawyer JS, Froelich LL, Mann LL, Spaethe SM. Blockade of human neutrophil activation by 2-[2-propyl-3-[3-[2-ethyl-4-(4-fluorophenyl)-5-hydroxyphenoxy]propoxy]phenoxy]benzoic acid (LY 293111), a novel leukotriene B4 receptor antagonist. *Biochem Pharmacol* 1995; **49**: 1683–1690
20 Schwartz G, Budman D, Endres S, Welch M, O'Reilly E, Barile-Thiem B, *et al.* Phase I and pharmacokinetic study of LY 293111, an orally available small molecule known to be an LTB4 receptor antagonist, 5-lipoxygenase inhibitor, and peroxisome proliferator activated receptor gamma agonist. *Proc Am Soc Clin Oncol* 2002; **21**:343a
21 Chou T, Talalay P. Generalized equations for the analysis of inhibitions of Michaelis–Menton and higher order kinetic systems with two or more mutually exclusive and non-exclusive inhibitors. *Eur J Biochem* 1988; **115**:207–216
22 Adams J. Potential for proteasome inhibition in the treatment of cancer. *Drug Disc Today* 2003; **7**:307–315
23 Gabriel B, Fischer DC, Kieback DG. Molecular mechanisms in signal transduction:new targets for the therapy of gynecologic malignancies. *Onkologie* 2002; **25**:240–247
24 Lu Y, Zi X, Zhao Y, Mascarenhas D, Pollak M. Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin). *J Natl Cancer Inst* 2001; **93**:1852–1857
25 Moasser MM, Basso A, Averbuch SD, Rosen N. The tyrosine kinase inhibitor ZD1839 ('Iressa') inhibits HER2-driven signaling and suppresses the growth of HER2-overexpressing tumor cells. *Cancer Res* 2001; **61**: 7184–7188
26 Nagy P, Jenei A, Damjanovich S, Jovin TM, Szolosi J. Complexity of signal transduction mediated by ErbB2: clues to the potential of receptor-targeted cancer therapy. *Pathol Oncol Res* 1999; **5**:255–271
27 Sinha S, Corey SJ. Implications for Src kinases in hematopoiesis: signal transduction therapeutics. *J Hematother Stem Cell Res* 1999; **8**:465–480
28 Ding XZ, Hennig R, Adrian TE. Lipoxygenase and cyclooxygenase metabolism: new insights in treatment and chemoprevention of pancreatic cancer. *Mol Cancer* 2003; **2**:10
29 Gupta RA, Tejada LV, Tong BJ, Das SK, Morrow JD, Dey SK, *et al.* Cyclooxygenase-1 is overexpressed and promotes angiogenic growth factor production in ovarian cancer. *Cancer Res* 2003; **63**:906–911
30 Singh B, Lucci A. Role of cyclooxygenase-2 in breast cancer. *J Surg Res* 2002; **108**:173–179
31 Stoehlmacher J, Lenz HJ. Cyclooxygenase-2 inhibitors in colorectal cancer. *Semin Oncol* 2003; **30**:10–16
32 Altorki N, Keresztes R, Port J, Libby D, Korst R, Flieder D, *et al.* Celecoxib, a selective cyclo-oxygenase-2 inhibitor, enhances the response to preoperative paclitaxel and carboplatin in early-stage non-small-cell lung cancer. *J Clin Oncol* 2003; **21**:2645–2650
33 Subbaranaiah K, Derrick T, Hart J, Dannenberg A. Peroxisome proliferator-activated receptor gamma ligands suppress the transcriptional activation of cyclooxygenase-2. *J Biol Chem* 2001; **276**:12440–12448
34 Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* 1998; **391**:79–82
35 Inoue H, Tanabe T, Umesono K. Feedback control of cyclooxygenase-2 expression through PPAR-gamma. *J Biol Chem* 2000; **275**:28028–28032.