

Development and Characterization of a Fixed Ratio Formulation of Cisplatin and Irinotecan: *In Vitro* and *In Vivo* Activity Against the H460 Human Lung Carcinoma Model

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Introduction

Drug combinations utilized in cancer chemotherapy regimens historically have been developed empirically by escalating the dose of the individual drugs to toxicity endpoints. The selection of drugs to combine have often been based on *in vitro* studies utilizing mathematical algorithms that can elucidate whether drugs combine in an additive, synergistic or antagonistic fashion. However, unlike *in vitro* assays where drug exposure can be readily controlled, in animal models the duration and extent of drug exposure to tumor cells is typically unknown and difficult to control. Consequently, a major challenge in the optimization of drug combinations has been the ability to capture the benefits of synergistic effects observed *in vitro* in the *in vivo* setting. Cisplatin and irinotecan (CDDP:CPT-11) are an effective drug combination, providing synergistic activity in tissue culture as well as improved treatment outcomes for patients with NSCLC. We have developed an approach whereby drug carriers are used to fix drug ratios and maintain these ratios after IV administration. This overcomes the problems associated with conventional drug combination “cocktails” where each agent distributes and is metabolized independently in the body, resulting in uncontrolled drug ratios *in vivo*. The goal of this research was to develop and characterize a single formulation comprising liposomal CDDP and liposomal CPT-11 mixed at fixed ratios and determine whether trends in synergy/antagonism observed in tissue culture were reflected *in vivo*.

Methods

MTT-viability assays done in triplicate were completed for each individual agent and for combinations of agents at fixed molar ratios (72-hour drug incubation). The averaged data was entered into the CalcuSyn program, developed from the quantitative analysis of dose-effect relationships developed by Chou & Talalay (Adv. Enzyme Reg. 22:27-55, 1984) for analysis of combined effects of multiple drugs. The median-effect analysis linearized the sigmoidal viability curves and provided calculated effective doses for individual and combined drugs, and assigned a combination index (CI) derived from the CI equation used to determine synergy. CI=1 is additive, CI>1 is antagonistic and CI<1 is synergistic. For the MTT assay, CI-values are most reliable between f_a 0.2 and 0.8.

Formulations containing different ratios of CDDP:CPT-11 were prepared by mixing liposomes with passively encapsulated CDDP (100 nm diameter DMPC:Chol (55:45 mol%)) and liposomes with actively encapsulated CPT-11 (preformed DSPC:DSPE-PEG₂₀₀₀ (95:5 mol%)) liposomes containing copper gluconate. Unencapsulated drugs were removed by dialysis. In the final formulations >95% of the drugs were inside the liposomes. The molar ratios could be varied between 1:10 and 10:1 by mixing the respective amounts of the individual liposomes prior to injection. Drug treatments were diluted in sterile saline for IV administration.

For PK analysis the formulations were administered IV into female Scid/Rag2 mice (4 per group). Blood was collected at indicated times in EDTA-treated tubes. Plasma was analyzed for total Irinotecan, CDDP, and liposomal lipid utilizing HPLC for irinotecan, atomic adsorption for CDDP and a non-exchangeable radiolabel marker ³H-CHE for liposomal lipid (data not shown). For therapeutic studies SCID/Rag2 mice bearing solid tumors (6 mice per group) were administered treatments IV on a q4d x 3 dosing schedule. Tumor measurements and body weights were taken 3x weekly. Study endpoints were determined when control tumors reached 1000 mg or when tumors became ulcerated. Calculation of quantitative treatment induced tumor growth delay parameters was based on the treatment induced time delay for tumors to reach 600% of initial volume and the tumor doubling time, which yielded the Log Cell Kill (equivalent to fraction effected). *In vivo* analysis for synergy/antagonism using the CalcuSyn program utilized dose response curves induced by the individual liposomal drugs in comparison of different fixed ratio formulations of CDDP:CPT-11.

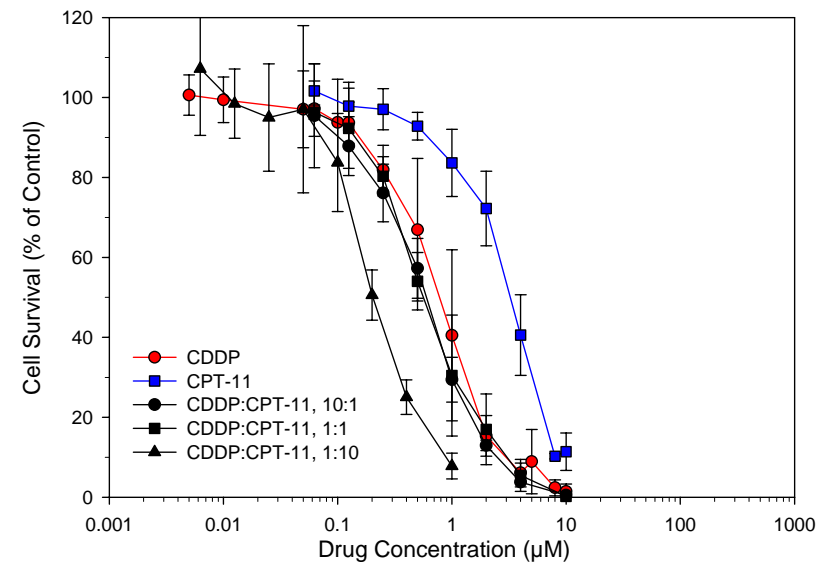


Figure 1: *In vitro* survival curves of CDDP, CPT-11 and combinations of CDDP:CPT-11 at molar ratios of 10:1, 1:1, and 1:10 in the H460 lung cancer cell line. Drug concentrations for combinations are based on CDDP.

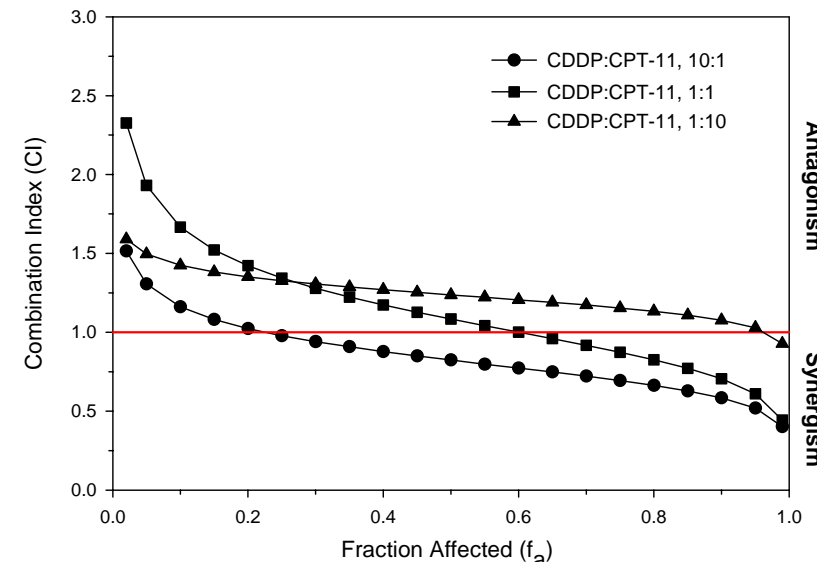


Figure 2: The *in vitro* viability in H460 cells of CDDP:CPT-11 combinations is dependent on the Fraction Affected (f_a , fraction of cells killed) and the CDDP:CPT-11 molar ratio.

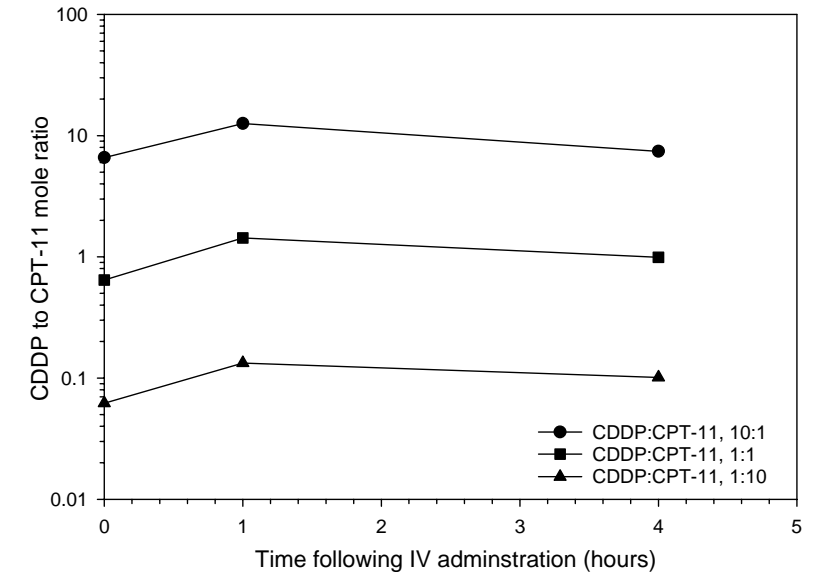


Figure 3: Formulated drug ratios can be maintained following IV administration of DMPC:Chol (55:45 mol%), liposomal CDDP, and DSPC:DSPE-PEG₂₀₀₀ (95:5 mol%) liposomal CPT-11 at fixed drug molar ratios of 1:10, 1:1, and 10:1.

Table 1: CI values obtained from *in vivo* efficacy experiments in the H460 Scid/Rag2 model for different fixed ratio liposome formulations of CDDP:CPT-11 compare favorably with CI values obtained in *in vitro* viability screening studies.

DRUG	DOSE (µmol/kg)	T-C ^a	% GROWTH DELAY ^b	LOG CELL KILL ^c	% CELL KILL ^d	CI (<i>in vivo</i>) ^e	CI (<i>in vitro</i>) ^f
L-CDDP + L-CPT-11 (1:10 mol ratio)	3.69 + 36.9	15.3	70.23	1.18	93.38	1.063	1.028
L-CDDP + L-CPT-11 (1:1 mol ratio)	3.33 + 3.33	2.8	12.97	0.22	39.45	1.512	1.173
L-CDDP + L-CPT-11 (10:1 mol ratio)	5 + 0.5	2.5	11.45	0.19	35.77	0.794	0.908

^a T - C = the difference in days for a treatment tumor to increase in volume by 600% compared to control tumors.
^b % growth delay = ((T - C)/C) x 100; where C is the day when control tumor reaches 600%.
^c Log cell kill = (T - C)/(3.32 x Td); where Td is the tumor doubling time of control tumors.
^d % cell kill = (1 - (1/10^X)) x 100; where X is the log cell kill.
^e CI (*in vivo*) represents the mutually-exclusive CI value determined using the CalcuSyn software (Median-effect principle of Chou and Talalay).
^f CI (*in vitro*) reports the simulated CI values for the indicated % cell kill (equivalent to the fraction affected (f_a)) computed by CalcuSyn software following analysis of H460 cell viability *in vitro* as a consequence of treatment with free cisplatin and free CPT-11 in combination at equivalent drug:drug molar ratios.

Conclusions

- Analysis of antitumor activity for synergy using the median-effect method of Chou and Talalay can be performed *in vivo* as well as *in vitro*, yielding Combination Index (CI) values which reflect synergy, additivity, or antagonism.
- CI values observed *in vivo* for fixed drug ratio liposome formulations correlated with CI values obtained *in vitro*.
- Liposome formulations containing fixed drug ratios allow *in vitro* synergy information to be translated *in vivo*.