

# Interaction between cisplatin, 5-fluorouracil and vincristine on human hepatoma cell line (7721) \*

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**Subject headings** liver neoplasms; cisplatin; 5-fluorouracil; vincristine; cell line

## Abstract

**AIM** To evaluate the killing effects of CDDP, 5-Fu and VCR on human hepatoma cell line (7721).

**METHODS** The median-effect principle was used.

**RESULTS** Killing effects of the individual drug were enhanced as the median concentration increased. Antagonism was produced when two drugs were used at a higher concentration ( $CI > 1$ ), and synergism was achieved when  $CI < 1$ . Finally, the effect was influenced by both the ratios of drug concentration and the sequence of administration.

**CONCLUSION** The drug administration order and drug concentrations are significant factors that need to be considered in clinical practice.

## INTRODUCTION

The combined chemotherapy for malignant carcinoma is desired to produce efficacious synergism between each drug, alleviate side effects of drugs and delay drug resistance. Clinically, the interaction (namely synergism, summation and antagonism) of different anticancer drugs in combination is usually evaluated by Chou-Talalay's combination index (i.e., median-effect principle)<sup>[1-9]</sup>. In this paper the combination effect between Cisplatin (Cis), 5-Fluorouracil (5-Flu) and Vincristine (VCR) on human hepatoma cell line 7721, was analyzed *in vitro*.

## MATERIALS AND METHODS

### Cell line and culture conditions

A human hepatoma cell line (7721 cells) was obtained from the Department of Bioengineering, Chongqing University. The cell line was cultured in RPMI 1640 (GIBCO, Grand Island, NY) with 10% (v/v) fetal calf serum, and 100 U/mL penicillin-streptomycin antibiotics, and kept at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>.

### Agents

Cis was purchased from Yunnan Gejiu Biochemical Pharmarcetics, 5-Fu from Tianjin Renming Pharmaceutical plant and VCR from Shanghai 11th Pharmaceutical Factory. These drugs were freshly prepared, dissolved in 0.9% NaCl solution before use, at concentrations of 40, 20, 10, 5, 2.5 mg/L for Cis, 5-Fu 400, 200, 100, 50, 25 mg/L, and VCR 4, 2, 1, 0.5, 0.25mg/L, respectively.

### Dose-effect test

The cytotoxicity of the drugs on human hepatoma cell line 7721 was examined by MTT assay<sup>[10]</sup>. Cells ( $1 - 5 \times 10^5$ /mL) were suspended in 10% FCS RPMI1640 medium and 200µl/well suspension was plated to 96 well microplate. Negative control and treatment groups were set up for each experiment.

### Effect of anticancer drug used alone or in combination

When the drug was used alone, each drug at five different concentrations were added to 96 well microplate at 20 µl/well in three duplicates. When used in pairs, Cis and 5-Fu, Cis and VCR, 5-Fu and VCR at a ratio of 1×1 were added to microplate at a total of 20µl/well (each drug 10µl at two times

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\*Project supported by Chinese Ministry of Public Health.

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Received 1998-05-25

concentration that of the drug used alone). Cells were incubated at 37°C, 5% CO<sub>2</sub> for 3 days.

#### Concentration dependence relationship in drugs used in combination

Cis at five different concentrations 2.5, 5, 10, 20 and 40 mg/L were combined with 5-Fu 100, 200 mg/L or VCR 1, 2 mg/L, respectively; VCR 0.25, 0.5, 1, 2 and 4 interacted with 5-Fu at 100 and 200 mg/L. The process lasted 3 days at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>.

#### Time-order dependence relationship in drugs used in combination

The time order for Cis and 5-Fu, Cis and VCR administration was as follows: two drugs were given simultaneously and cells were cultured for 3 days; and one drug was given first, another was then administered, and cells incubated for 2 days.

#### Data analysis

OD values of control and treatment groups were detected by Bio-Rad E2550 EIA Reader. Cytotoxicity = 1 - (mean OD of treatment group/mean OD of control group).

According to the median equation  $fa/fu = (D/D_m)^m$ , the median effect curve,  $Y = bx + a$  ( $Y = \log fa/fu$ ,  $x = \log D$ ) was plotted.  $D$  is the dose,  $D_m$  is the median effect dose required for 50% cytotoxicity,  $fa$  represents the fraction affected by the dose  $D$ ,  $fu$  defined the fraction unaffected equals  $1-fa$ , and  $m = b$  is the slope. The median effect dose is calculated by  $\log D_m = -a/m$ ,  $a = Y - bX$ . The interaction of two drugs is determined quantitatively by combination index (CI):

$$CI = (D_1)^x / (D_x)_1 + (D_2)^x / (D_x)_2 + \alpha (D_1)^x (D_2)^x / (D_x)_1 (D_x)_2$$

$D_1$  and  $D_2$  are the doses of drug 1 and 2, which are required to produce  $x\%$  effect in combination.  $(D_x)_1$  and  $(D_x)_2$  are the dose of drug 1 and drug 2 required to produce  $x\%$  effect individually. If the drugs are mutually exclusive,  $\alpha = 0$  and non-exclusive,  $\alpha = 1$ .  $CI = 1$ , summation;  $CI < 1$ , synergism; and  $CI > 1$ , antagonism<sup>[11]</sup>.

## RESULTS

#### Dose-effect relationships of three drugs used alone and in combination

The cytotoxicity of three drugs progressively increased with increasing doses, either used alone or in pairs (Table 1). According to the median-effect equation, the median effect doses of these drugs used alone or in combination can be calculated. When used alone, Cis was 1.158 mg/L, 5-Fu 17.056 mg/L and VCR 5.22 mg/L, respectively. In contrast to the drug used alone, the median-effect doses were lower in combination. Cis plus VCR was

0.85 mg/L (containing Cis 0.82 mg/L and VCR 0.03 mg/L); Cis plus 5-Fu 7.43 mg/L (containing Cis 0.31 mg/L and 5-Fu 7.21 mg/L); and 5-Fu plus VCR was 8.45 mg/L (containing 5-Fu 8.44 mg/L and VCR 0.01 mg/L). The combined effect of two drugs at ratio 1:1 can be illustrated for synergism, summation or antagonism (Figure 1).

Table 1 Dose-effect relationships of three drugs used alone and in combination

Cis	5-Fu	VCR	Cis+5-Fu	VCR+5-Fu	Cis+VCR
(2.5)0.662	(25)0.600	(0.25)0.178	(2.5+25)0.709	(2.5+25)0.647	(2.5+25)0.527
(5.0)0.809	(50)0.784	(0.50)0.204	(5+50)0.859	(5+50)0.713	(5+50)0.781
(10.0)0.867	(100)0.834	(1.00)0.282	(10+100)0.877	(10+100)0.810	(10+100)0.844
(20.0)0.893	(200)0.853	(2.00)0.429	(20+200)0.902	(20+200)0.839	(20+200)0.873
(40.0)0.928	(400)0.871	(4.00)0.446	(40+400)0.951	(40+400)0.892	(40+400)0.898

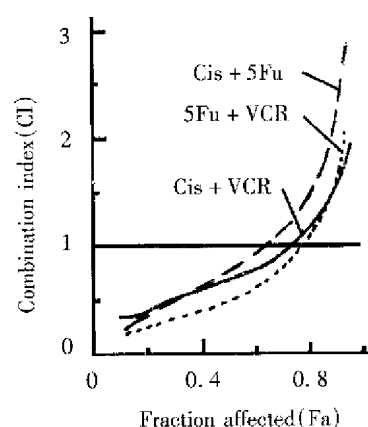


Figure 1 The relationship between CI and fraction affected of Cis+5-FU, VCR+5-Fu and 5-Fu+VCR.

#### Concentration dependence relationship in combination

**Cis and 5-Fu** The interaction of 5-Fu at a dose of 200 mg/L and Cis at five concentrations exhibited antagonism, the same results can be seen when 5-Fu at 100 mg/L was combined with Cis at  $\geq 20$  mg/L, but synergism was shown when Cis at 20 mg/L.

**Cis and VCR** Most cases in which Cis at various concentrations and VCR at 1 mg/L (except for Cis at 2.5 mg/L), were used, showed antagonism. When Cis at various concentrations (except for 2.5 mg/L) was combined with VCR at 2 mg/L, synergistic effect appeared.

**VCR and 5-Fu** The combination of VCR at  $> 0.5$  mg/L and 5-Fu at 100 mg/L showed synergism. In contrast, when VCR at 0.5 mg/L or  $< 0.5$  mg/L and 5-Fu 100 mg/L, were used in combination antagonism occurred. Moreover, when 5-Fu at up to 200 mg/L was combined with Cis at various concentrations (except for Cis. the highest one) the interaction was also antagonism (Table 2).

**Table 2 Fa and CI of drug combinations at different ratios**

Drug 1 (mg/L)	Drug 2 (mg/L)	fa	CI	Drug 1 (mg/L)	Drug 2 (mg/L)	fa	CI
Cis 2.5	5-Fu 100	0.821	0.75	Cis 2.5	VCR 2	0.554	1.15
5	100	0.856	0.85	5	2	0.858	0.80
10	100	0.877	0.87	10	2	0.862	0.74
20	100	0.883	1.09	20	2	0.873	0.71
40	100	0.893	1.23	40	2	0.904	0.66
Cis 2.5	5-Fu 200	0.839	1.85	VCR 0.25	5-Fu 100	0.657	2.39
5	200	0.867	2.93	0.5	100	0.761	1.15
10	200	0.899	2.95	1	100	0.810	0.77
20	200	0.902	3.00	2	100	0.826	0.64
40	200	0.907	3.36	4	100	0.850	0.51
Cis 2.5	VCR 1	0.498	0.32	VCR 0.25	5-Fu 200	0.759	2.13
5	1	0.825	1.38	0.5	200	0.783	1.79
10	1	0.844	2.05	1	200	0.825	1.28
20	1	0.852	2.09	2	200	0.839	1.10
40	1	0.894	3.40	4	200	0.867	0.92

As can be seen in Table 2, the more prominent efficacy can be achieved when 5-Fu at lower concentration was used with other two drugs, while VCR could be used at higher concentrations.

#### Time-order dependence relationship in drugs used combination (Table 3)

**Cis and 5-Fu** When Cis and 5-Fu were used simultaneously, the effect was more efficacious than that of Cis used for 24h followed by 5-Fu or 5-Fu followed by Cis (*t* test,  $P < 0.05$ ). In contrast, there was no difference between Cis and 5-Fu no matter which was used first ( $P > 0.05$ ).

**Cis and VCR** When Cis was given 24h after VCR, the fraction affected was less prominent than that of two drugs treated simultaneously or Cis first ( $P < 0.05$ ). There was no apparent difference between two drugs used simultaneously and Cis used first ( $P > 0.05$ ).

**Table 3 Relationship between time-order and effect in drugs used in combination ( $\bar{x} \pm s$ )**

Drugs (mg/L)	Simultaneous exposure	Pre-Cis exposure	Post-Cis exposure
<b>Cis+5-Fu</b>			
2.5 25	0.709±0.015	0.683±0.016	0.598±0.010
5 50	0.859±0.011	0.807±0.027	0.773±0.015
10 100	0.877±0.013	0.824±0.012	0.793±0.026
20 200	0.902±0.014	0.831±0.017	0.805±0.016
40 400	0.951±0.012	0.853±0.015	0.822±0.010
<b>Cis+VCR</b>			
2.5 0.25	0.527±0.025	0.470±0.023	0.199±0.023
5 0.5	0.781±0.034	0.707±0.022	0.407±0.017
10 1	0.844±0.015	0.789±0.025	0.533±0.013
20 2	0.873±0.017	0.818±0.029	0.651±0.021
40 4	0.898±0.025	0.893±0.020	0.752±0.025

## DISCUSSION

Cis, a broad spectrum in anti-cancer drug, inhibits RNA and protein synthesis of tumor cells by cross-

linking with DNA. 5-Fu, an antimetabolic agent, halts DNA and RNA synthesis by inhibiting the activity of thymine nucleotide synthetase at various times in the cell cycle, especially in S phase. Thus, the common mechanism of the two drugs Cis and 5-Fu can stop RNA synthesis. VCR can inhibit cell microtubule integrity and therefore stops nucleospindle formation in cell division. *In vitro*, these three drugs exhibited pronounced cytotoxicity when used alone, in human hepatoma cell line 7721, but higher doses were needed. Compared with the drugs used alone, both the median effect concentration and the dose required were much lower when used in combination. The results indicate that lower dose in combination can achieve higher efficacy with less side-effects. When used in combination, the time order of administration affected drug cytotoxicity. If the cytotoxicity of the drug was pronounced when used alone, there was no difference when used in combination disregarding which was administered first. If one drug efficacy is more prominent than another, when used alone a higher combined effect can be achieved when the more efficacious drug was used first.

Anticancer drug sensitivity can analyze quantitatively the drug interaction, i.e. synergism, summation and antagonism, and hence guiding the clinical treatment.

**ACKNOWLEDGEMENT** We would like to thank Professor FAN Wei-Ke for preparing the manuscript.

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