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Synergistic anticancer effects of tetrandrine combined with doxorubicin or vincristine *in vitro*[1]

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KEY WORDS tetrandrine; doxorubicin; vincristine; multiple drug resistance; cultured tumor cells; drug synergism; drug combinations; tetrazolium salts

ABSTRACT

AIM: To study the interaction between tetrandrine (Tet) and doxorubicin (Dox) or vincristine (Vin) against human breast cancer cell lines MCF-7 and MCF-7/Dox or human nasopharyngeal cancer cell lines KB and KB_{V200} *in vitro*. **METHODS**: Anticancer activities of a drug alone and a drug combination of Tet and Dox or Vin were determined by tetrazolium (MTT) method. The interaction between Tet and Dox or Vin was evaluated by both a value of a sum of fractional inhibitory concentration (SFIC) and an isobologram method. **RESULTS**: The SFIC values of the three-different-ratio combinations between Tet and Dox ranged from 0.14 to 0.38 for MCF-7, and 0.10 to 0.29 for MCF-7/Dox; those of Tet-Vin combinations ranged from 0.21 to 0.37 for KB, and 0.32 to 0.63 for KB_{V200}. All the SFIC values of the combination between Tet and Dox or Vin were less than 1.0 when the 3 ratios of the 2 drugs in combination were used, and the shapes of isobolic curves obtained from the combination were concave. **CONCLUSION**: The interaction between Tet and Dox or Vin against the human cancer cells was markedly synergistic.

INTRODUCTION

Multidrug resistance (MDR) of carcinoma cells is one of the major factors leading to chemotherapeutic failure. Searching for potentiating agents, which can increase the antitumor efficacy of a drug, so much as to circumvent the resistance, may be another avenue to overcome MDR. Tetrandrine (Tet) exhibited a marked selective anticancer activity and remarkable reversal effects against doxorubicin (Dox)- or vincristine (Vin)-resistant human cancer cells MCF-7/Dox or KB_{V200}[1]. This paper was to study the nature of the interaction between Tet and Dox or Vin against human breast cancer cells MCF-7 and MCF-7/Dox or human nasopharyngeal cancer cells KB and KB_{V200}, respectively.

MATERIALS AND METHODS

Drugs and reagents Dox hydrochloride for injection (10 mg/vial, lot № 970203, Zhejiang Haimen Pharmaceutical Factory) or Vin sulfate for injection (1 mg/ampoul, lot № 961101, Experimental Pharmceutical Factory of Beijing Medical University) solutions were prepared using normal saline and filtered. Tet powder with a purity of 96.5 %, a raw material for producing Tet tablets (Zhejiang Jinhua Pharmaceutical Factory) was dissolved in an acid solution and then pH was adjusted to 6.0. A series of dilutions was prepared just before test.

Cell culture MCF-7 and MCF-7/Dox

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(NCI, USA) were routinely cultured in Dulbecco's minimal essential medium (DMEM, Gibco) containing 15 % bovine serum at 37 ℃ in 95 % air + 5 % CO_2[2]. KB and KB_{V200} cells from Institute of Materia Medica, Chinese Academy of Medical Sciences were cultured in RPMI-1640 medium (Gibco) containing 15 % bovine serum at 37 ℃ in 95 % air + 5 % CO_2[3].

Measurement of anticancer activity The tetrazolium (MTT) assay system used here was essentially similar to the reported one[4]. Briefly, the cell suspension ($4 \times 10^7 - 1 \times 10^8$ cells·L^{-1}) was added into 96-well tissue culture plates (100 μL /well) and incubated overnight. The cells were then exposed to 200 μL of various concentrations of a drug alone or a drug combination for 72 h. After that, an MTT assay was conducted in the same pattern[1], and the IC_{50} (95 % confidence limits) were calculated by weighted probit analysis (Bliss and Finney).

Interaction of drug combination Based on IC_{50} values of an individual drug and the ratio of the IC_{50} values between the 2 drugs in a preliminary experiment, Tet and Dox or Vin were mixed in fixed ratios[5]. For example, in a preliminary experiment, the ratios of the IC_{50} values of Tet *vs* Dox against MCF-7/Dox was found to be about 1∶3, therefore, a ratio of the 2 drugs in a mixture was selected as 1∶3 usually referred to as Group 2, and then the proportion of one drug in the combination was multiplied by 3.0, while that of the other remained unchanged. As a result, a total of 3 different ratios for a certain drug-combination (for instance, 3∶3, 1∶3, and 1∶9 for Tet + Dox) were obtained. The 3 ratios were designated as combination group 1, 2, and 3. The IC_{50} values of each drug used alone and an individual drug in the combination group 1, 2, and 3 were calculated using probit analysis. Each combination group had 2 IC_{50} values; one was for Tet, and the other for Dox or Vin. Sum of fractional inhibitory concentration (SFIC) of the drug combination was calculated by the following equation: SFIC = IC_{50} of one drug in the combination/IC_{50} of the corresponding drug used alone + IC_{50} of the other drug in the combination/IC_{50} of the corresponding drug used alone. If the SFIC value was less or more than 1.0, or equal to 1.0, potentiating or antagonistic, or additive interaction was thought to occur. In addition the isobolograms were constructed[6]. Tet FIC (IC_{50} of Tet in the combination/IC_{50} of Tet alone) was designated as a value on the abscissa of an isobolic figure, while FIC of the other drug (Dox or Vin) in the combination (IC_{50} of Dox or Vin in the combination/IC_{50} of Dox or Vin alone) as a value on the ordinate. When the isobolic curve was concave or convex, the nature of the interaction between the 2 drugs in combination was considered to be synergistic or antagonistic.

Triplicate wells were used for each drug concentration. Experiments were triplicated.

RESULTS

Inhibition of cell growth by one drug alone The IC_{50} values ofTet were much greater than those of Dox and Vin against the 2 sensitive cancer cell lines, MCF-7 and KB cells. The IC_{50} values of Tet against MCF-7/Dox and KB_{V200} cells were less than those against MCF-7 and KB cells ($P > 0.05$). (Tab 1, 2)

Inhibition of cell growth by drug combination When Dox or Vin was combined with Tet, IC_{50} values of Dox or Vin in the combination group 1, 2, and 3 were lower than that of Dox or Vin alone, regardless of which line of cancer cells was used. In general, IC_{50} value of Dox or Vin was decreased with increasing dose of Tet in the combination. With the 4 human cancer cells used in this study, the SFIC values of all the combinations between Tet and Dox or Vin were less than 1.0, and the shapes of isobolograms for

Tab 1. IC_{50} of Tet or Dox or Tet + Dox against MCF-7 and MCF-7/Dox *in vitro*. The ratios of Tet *vs* Dox in group 1, 2, and 3, were 192:1, 64:1, and 64:3 in MCF-7, and 1:1, 1:3, and 1:9, in MCF-7/Dox, respectively. n = 3 independent experiments. 95 % Confidence limits are in parentheses.

Drug		IC_{50} against cancer cells, $\mu mol \cdot L^{-1}$			
		MCF-7		MCF-7/Dox	
Tet		6.080	(0.190 - 11.980)	5.610	(1.192 - 10.028)
Dox		0.067	(0.050 - 0.084)	4.070	(0.660 - 7.480)
Tet + Dox					
Group 1	Tet	2.110	(-0.722 - 4.942)	0.150	(0.104 - 0.196)
	Dox	0.011	(-0.004 - 0.026)	0.150	(0.104 - 0.196)
Group 2	Tet	0.842	(0.074 - 1.610)	0.151	(0.052 - 0.250)
	Dox	0.013	(0.001 - 0.025)	0.475	(0.163 - 0.787)
Group 3	Tet	0.127	(-0.004 - 0.258)	0.073	(-0.018 - 0.164)
	Dox	0.006	(0 - 0.012)	0.624	(-0.154 - 1.402)

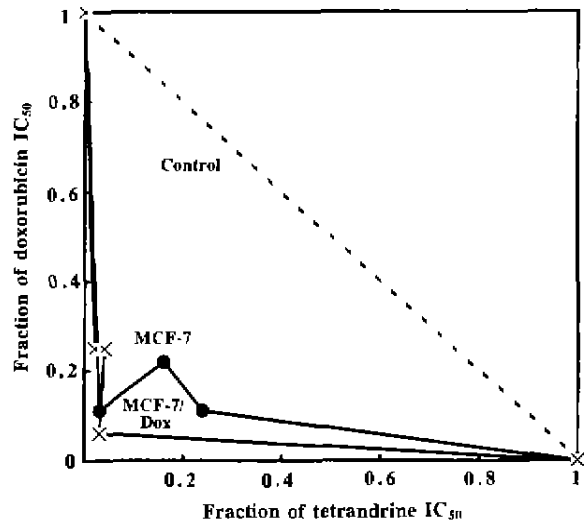

Fig 1. Isobolograms of anticancer interaction between Tet and Dox against MCF-7 and MCF-7/Dox *in vitro*.

Tab 2. IC_{50} of Tet ($\mu mol \cdot L^{-1}$) or Vin ($nmol \cdot L^{-1}$) or Tet + Vin against KB and KB_{V200} *in vitro*. The ratios of Tet *vs* Vin in group 1, 2, and 3, were 27 000:1, 9000:1, and 3000:1, respectively in KB, and 6:1, 2:1, and 2:3, in KB_{V200}, respectively. n = 3 independent experiments. 95 % Confidence limits are in parentheses.

Drug		IC_{50} against cancer cells			
		KB		KB_{V200}	
Tet		10.180	(4.516 - 15.811)	4.480	(-0.454 - 9.414)
Vin		9.190	(-6.110 - 24.490)	5.190	(-27 - 10.407)
Tet + Vin					
Group 1	Tet	2.077	(-0.286 - 4.440)	1.430	(-0.096 - 2.946)
	Vin	0.075	(-0.009 - 0.165)	234	(-31 - 499)
Group 2	Tet	2.630	(0.265 - 4.995)	0.851	(-0.442 - 2.144)
	Vin	0.301	(0.041 - 0.561)	424	(-229 - 1077)
Group 3	Tet	1.820	(0.604 - 3.037)	0.691	(-0.572 - 1.954)
	Vin	0.570	(0.177 - 9.963)	994	(-894 - 2882)

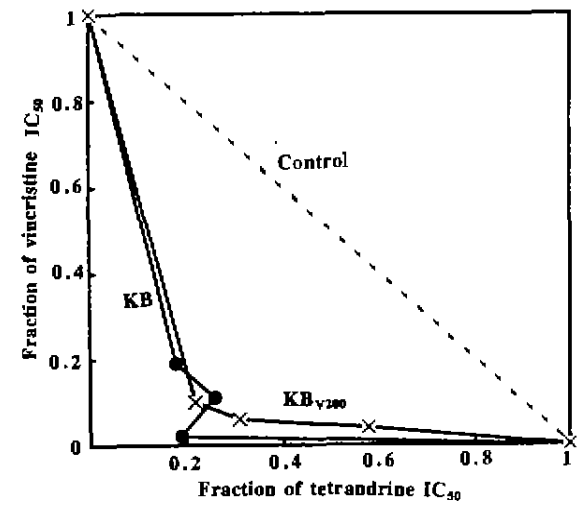

Fig 2. Isobolograms of anticancer interaction between Tet and Vin against KB and KB_{200} *in vitro*.

the combinations between Tet and Dox or Vin were concave (Tab 3, Fig 1, 2).

DISCUSSION

Both the SFIC values and the shapes of isobolic curves indicated a potentiating interaction between Tet and Dox or Vin in the combination against the 4 human cancer cells *in vitro*. This synergistic interaction was also found in the combination of Tet and chloroquine against *Plasmodium falciparum in vitro*[7].

The mechanism of Tet potentiating antitumor activity may be different from that of Tet reversal effect on the resistance. The latter pointed towards the resistant cancer cells and might be somehow related to operation of MDR, such as

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Tab 3. Effects of Tet + Dox or Tet + Vin on cancer cells MCF-7 and MCF-7/Dox or KB and KB_{V200} *in vitro*. $\bar{x} \pm s$. The ratios of Tet/Dox or Tet/Vin in group 1, 2, and 3 are identical to those in Tab 1, 2. S and R lines represent MCF-7 and MCF-7/Dox in the combination of Tet and Dox, or KB and KB_{V200} in the combination of Tet and Vin, respectively. SFIC: a sum of fractional inhibitory concentration.

Cancer cells	Trial	SFIC of Tet + Dox			SFIC of Tet + Vin		
		Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
S line	1	0.62	0.20	0.04	0.07	0.13	0.40
	2	0.41	0 79	0.30	0.26	0.65	0.47
	3	0.03	0.14	0.09	0.30	0.33	0.24
		0.35 ± 0.30	0.38 ± 0.36	0.14 ± 0.14	0.21 ± 0.12	0.37 ± 0.26	0 37 ± 0.12
R line	1	0.18	0.61	0.57	0.92	0.74	0.88
	2	0.04	0.20	0.01	0.13	0.06	0.04
	3	0.07	0.04	0.23	0.83	0.30	0.03
		0.10 ± 0.07	0.29 ± 0.29	0.27 ± 0.28	0.63 ± 0.43	0.37 ± 0.34	0.32 ± 0.49

over-expression of P-gp[8]. However, Tet manifested the potentiating effects against the sensitive cancer cells as well as the resistant ones. The sensitive cancer cells have nothing to do with MDR. Therefore, involvement of MDR can not become a convincing explanation for the mechanism of Tet potentiating activity, at least for the sensitive cells. Once enhancement of antitumor potency of a drug by a potentiating agent like Tet dominated MDR of resistant cancer cells, MDR was overcome. In this case, the mechanism of the potentiating effect by the agent may be or may not be associated with MDR operation in the resistant cells.

In conclusion, the nature of the interaction between Tet and Dox or Vin is markedly synergistic.

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粉防己碱与阿霉素或长春新碱联合用药的体外抗癌增效作用[1]

R730.53 R979.1

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关键词 粉防己碱; 阿霉素; 长春新碱;

多种抗药性；培养的肿瘤细胞；药物协同作用；合并用药；四唑铵盐

目的：研究粉防己碱(Tet)和阿霉素(Dox)或长春新碱(Vin)联合用药在体外分别对人乳腺癌细胞MCF-7及其抗Dox细胞MCF-7/Dox或人口腔上皮样癌细胞KB及其抗Vin细胞KB_{V200}的相互作用的性质. **方法**：MTT法测定Tet和Dox或Vin单独用药或联合用药的抗癌活性. 抑制浓度的分数之和(SFIC)值及等效剂量分析方法(Isobologram)评价Tet和Dox或Vin联合用药的相互作用的性质. **结果**：Tet和Dox或Vin联合用药时分别以三种不同比例配伍，其SFIC值均小于1. 由此联合用药得到的等效剂量曲线图形均为凹形. **结论**：Tet和Dox或Vin体外联合用药抗癌相互作用的性质为明显的增效作用.

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