Effects of Kanglaite Injedction on Reversing Multiple Drug Resistance (MDR) of Tumor Cells

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Abstract

Multiple drug resistance (MDR) is one of the main causes leading to the failure of cancer chemotherapy. MDR reversing agents have broad prospect in clinical applications. This study investigates the effects of Kanglaite Injection (KLT) on MDR cancer cells by examining the sensitivity of drug resistant human erythroleukemia cell strain K562/vcr to KLT and a variety of chemotherapeutic agents. MTT method was applied in the research. Using MDR reversing agent VRP as control, we studied the effects of KLT on tumor cell sensitivity to Doxorubicin (DOX). The study showed that the sensitivity of the drug resistant cell strain K562/vcr to KLT is below that of the drug sensitive cell strain K562/vcr. The resistance refractory was 3.54. An experiment on reversing the MDR of K562/vcr cell strain demonstrated that the resistance modification index (RMI) of VRP 6μg/ml was 125 and RMI of KLT 8 μl/ml was 54.1. Our study suggested that KLT was effective in reversing MDR of cells and increasing the sensitivity of cancer cells to chemotherapeutic agents.
Key words: KLT, MDR

MDR is one of the main causes leading to the failure of cancer chemotherapy. The development of MDR is related to the high expression of P-Glyoprotein on the surface of tumor cell membrane. P-gp can expel drug already entered into cells out of the cells. As a result dosage of drug inside cells is reduced and MDR develops. MDR reversing agents have the action of suppressing P-gp and restoring sensitivity of MDR cancer cells to chemotherapeutic agents. There is a broad prospect in clinical application for MDR reversing agents. Many researchers have reported that VRP, when used together with chemotherapeutic agents, is very effective in reversing MDR. However clinical application of VRP is greatly restricted due to its toxicity. VRP to reverse MDR is dose-dependant. The maximum tolerance dose for VRP clinical application is 2umol/L, but the concentration for VRP to achieve its best MDR reversing effect is 3-6umol/L. So scientists are looking for other MDR reversing agents with high effect but low toxicity. Many pharmacological and clinical studies have demonstrated that KLT when combined with chemotherapy and radiotherapy could create a synergistic action, increase therapeutic effects and prevent and reduce chemotherapeutic and radiotherapeutic toxicity. The mechanism behind this action remained uncertain. This study investigated the effects of Kanglaite Injection on MDR cancer cells.

1. Materials and Methods

1.1 Cell culture and MDR cell strain

Human erythroleukemia K562 cell strain was provided by the Immunology Institute, Kiel

University, Germany. MDR K562/vcr cell strain was established by Dr. Hu Xun of Zhejiang Medical University Cancer Institute. The cells were cultured and passaged by the Department of Cytology of the institute.

1.2 Drugs and reagents

Kanglaite Injection (10%) was provided by Zhejiang Kanglaite Pharmaceutical Co., Ltd. Doxorubicin (DOX), epirubicin (EPI), daunorubicin (DNR), harringronine (HAR), mitomycin C (MMC), verapamil (VRP), methotrexate (MTX), [3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H -tetrazolium-bromide] (MTT) were purchased from Toubin, Farmitalia Carlo Erba, Minsheng, Kyowa and Serva companies respectively.

1.3 Instruments

Carbon dioxide cell incubator, WJ-RF
Inverted microscope, Olympus
Full automatic enzyme labeling photometer, Sigma 960

1.4 Cell drug resistance experiment and MDR reversing test (MTT Assay)

Logarithmic growth phase tumor cells 2×10^4/ml were inoculated into each well of a 96-well microculture plate. Anti-cancer agents with different concentration were added and the cells were cultured for 72 hours. MTT (1mg/ml) 50μl was added into each well. The cells were kept at 37℃ for 4 hours. The cell suspension was centrifugalized at 2,000 rpm for 10 minutes. The supernatant was discarded. 100μl acidulated isopropanol was then added into each well. After the crystalline dissolved completely, colorimetry was performed at 570nm wave length and the 50% inhibitory concentration (IC_{50}) was calculated. The IC_{50} for drug resistant cell strain was calculated according to the following formula.

Resistant Factor (RF)=Drug resistant cell strain IC_{50} / Drug sensitive cell strain IC_{50}

The method for cell drug resistance experiment was also applied to MDR reversing experiment. MDR reversing agents and doxorubicin were added at the same time into wells for drug resistant cell strain. The resistance modification index (RMI) was calculated according to the following formula.

RMI = IC_{50} of doxorubicin alone / IC_{50} of doxorubicin combined with MDR reversing agents

2. Results

2.1 The drug sensitivity of K562 cells and K562/vcr cells

Table 1 showed that K562/vcr cells were evidently resistant to either phytopharmaceuticals (VCR. HAR) or anthracyclines (DOX, EPI) and antibiotics (MMC). The K562/vcr cells were

also slightly resistant to KLT.

Table 1. Sensitivity of K562 and K562/vcr cells to anticancer agents

Agent	IC_{50}(μg/ml)		RF
	K562	K562/vcr	
DOX	0.9	30	33.3
EPI	0.8	20	25
DNP	0.6	2.5	4.2
VCR	<0.0012	4	3333
HAR	0.064	20	31
MMC	4	18	4.5
MTX	20	20	1
KLT	38.5μl/ml	136.4μl/ml	3.54

2.2 The effect of KLT on reversing MDR

Table 2 showed that VRP had the action of reversing MDR of K562/vcr cells. Within effective concentrations of VRP 1.5-6 μg/ml it had strong MDR reversing effect.

Table 2. The effect of VRP on reversing MDR of K562/vcr cells

DOX(mg/ml)	VRP(μg/ml)		
	6	3	1.5
0	0.817	0.808	0.802
0.01	0.809	0.889	0.703
0.1	0.458	0.512	0.639
1	0.146	0.181	0.1
10	0.147	0.189	0.190
DOX + VRP			
IC50*	0.24		0.546
RMI	125	0.285	55
		105.3	

*IC_{50} of DOX for K562/vcr was 30μg/ml.

Table 3 showed the effects of KLT on reversing the MDR of K562/vcr cells, indicating that at the concentration between 2-8 μl/ml, KLT had evident action on reversing MDR.

Table 3. The effect of KLT on reversing MDR of K562/vcr cells

DOX(mg/ml)	KLT(μg/ml)		
	6	3	1.5
0	0.846	0.765	0.805
0.01	0.692	0.718	0.782
0.1	0.686	0.701	0.804
1	0.166	0.192	0.207
10	0.086	0.141	0.174
DOX + KLT			
IC50		0.664	0.705
RMI	0.555	45.2	42.6
	54.1		

Figure 1 also shows the effects of KLT when used together with DOX on reversing MDR of K562/vcr cells.

3. Discussion

Drug resistance of cancer cells to chemotherapy agents is a major obstacle in cancer management. Although chemotherapy is initially effective in many cases, it remains quite hard to prevent cancer from recurrence due to development of MDR. Drug resistance can happen to many chemotherapeutic agents including alkylate agent, plant alkaloid, antibiotics and hormonal agents. Most of these drugs are composed of lipophilic macromolecular compound. When drug resistance occurs in cancer cells for one agent above, cross-resistance to all other agents usually develops at the same time.

When KLT is applied to patients with MDR the dosage of the drug should be increased if needed. This study demonstrated that KLT could apparently reverse MDR in cancer cells to those chemical agents by a quite small effective dosage (2-8μl/ml) which was far below its IC_{50} for K562/vcr cells. This might explain the mechanism behind the action of KLT to increase the sensitivity and efficacy in cancer treatment when combined with chemotherapeutic agents. This study suggested that KLT should be combined with chemotherapy in treating tumor patients with MDR in order to restore and increase the sensitivity of cancer cells to chemotherapy agents.

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