Effects of Kanglaite Injection on Cancer Cell Proliferation Cycle

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Abstract

The effects of Kanglaite Injection (KLT) on cancer cell proliferation cycle were investigated. The study revealed that blank emulsion had slight inhibitory effect on cancer cells. The inhibitory rate of 1:20 diluted emulsion (equal to 50μl/ml) for KB cells is 10%. The IC_{50} of KLT was 22.6μl/ml for KB cells and 38.5μl/ml for K562 cells. It has been observed with flow cytometric scan method that KLT suppresses cancer cell proliferation by inhibiting cell mitosis. After the cancer cells were treated with KLT, the number of S-phase cells decreased and the number of G_2+M phase cells increased with a dose dependent relationship. The proliferation ability of affected cancer cells was reduced, which eventually induced cancer cells into apoptosis.

Kanglaite Injection is produced from the active ingredient extracted by modern technology from Coix seed, a Traditional Chinese medicine. As an emulsion for injection, KLT has been proved by pharmacodynamic and clinical investigations as being of definite inhibiting action and therapeutic effects on many tumors. Experiments also showed that KLT could enhance human immune function. Investigating the mechanism underlying the anti-cancer action of KLT would serve as an important guidance for clinical practice. In this study, the effects of KLT on cancer cell proliferation cycle were investigated.

Key words: KLT, tumor cell, proliferation cycle

1. Materials and Methods

1.1 Drugs and Reagents

Kanglaite Injection (10%) and emulsion for control were supplied by Zhejiang Kanglaite Pharmaceutical Co., Ltd., Blue tetrazolium [3(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H tetraxolium-hrimide, MTT], RPMI 1640 and Propidium Iodide (PI) were purchased from Merck, Sigma and Gibco company respectively. Mitomycin (MMC) was produced from Kyowa.

1.2 Cell Culture

Human oral squamous carcinoma KB cell strain was supplied by Shanghai Biology Institute affiliated to the Chinese Academy of Science. Human erythroleukemia K562 cell strain was provided by the Immunology Institute of Kiel University in Germany. The cells were routinely cultured and passaged by the Department of Cytology of Cancer Institute of Zhejiang Medical University.

1.3 Instruments

Flow cytometer (FAC Scan) was purchased from Becton Dickinson, U.S.A. Full automatic

enzyme labeling photometer: Type of Sigma 960.

1.4 Methyl Thiazole Tetrazolium Reduction Method (MTT Method)
Logarithmic growth phase tumor cells 2×10^5/ml 0.2ml were inoculated into each well of a 96-well culture plate. Saline control group (well), Mitomycin (MMC) positive control group (well) and experiment group (well) of different concentrations were established with 6 parallel wells for each group. The cells were cultured in an incubator at 37℃, 5% CO_2 for 72 hours. MTT solution (5mg/ml) 10μl would be added into each well 4 hours before termination of the experiment. After the crystal dissolved completely, the absorption (O.D. value) of each well would be measured with enzyme labeling photometer at 570nm-wave length. The cell proliferation inhibitory rate was calculated with the following formula:

Inhibitory Rate (%)=(1- mean OD of experiment group/mean OD of control group)×100%.

The IC_{50} was calculated with logarithmic probability graphic method.

1.5 Analysis of Cell Proliferation Cycle-Flow cytophotometry (FCM)
The collected cells were fixed with dehydrated alcohol. Then treated them with RNase and stained with PI for a half hour before assay. Flow cytometric scan (FAC Scan) would be used for DNA assay. The data was processed by professional software Multicycle to calculate the percentage of cells of different phases.

2. Conclusions and Discussions

2.1 The Inhibitory Effects of KLT on Proliferation of Tumor Cells
The proliferations of KB and K562 cells got significant inhibition after treated with KLT for 72 hours. The IC_{50} of KLT was 22.6μl/ml for KB cells and 38.5% μl/ml for K562 cells. The inhibiting rate of 1:20 diluted control emulsion (equal to 50μl/ml) was 10% for KB cells and the IC_{50} of positive control Mitomycin (MMC) was 0.54 μg/ml for KB cells.

2.2 The Effects of KLT on Cancer Cell Proliferation Cycle
The following table showed that after treated by 1μl/ml KLT for 48 hours, the cells in S phase and G_2+M phase increased markedly in percentage. When the cells were treated by KLT at the concentration of 5μl/ml, the percentage of K562 cells in S phase decreased and the percentage of cells in G_2+M phase increased notably. With KLT of 10μl/ml, the K562 cells of S phase in experiment group decreased to 11.6% of those in control group while the cells of G_2+M phase in experiment group increased to 11 times of those in control. When the cells were treated by KLT at the concentration of 50μl/ml, 100% cells would be killed and no phase distribution of cell proliferation cycle could be analyzed. The experiment above showed that the proliferation cycle of K562 cells could be notably affected by KLT and that there existed dose dependent relationship. The action of KLT on cancer cell proliferation cycle was mainly due to its effects of retarding the cell cycle at the G_2+M phase, reducing the number of cells entering into G_0 and G_1 phase and decreasing the percentage of S-phase cells. As a result KLT

could inhibit mitosis and proliferation of cancer cells and induce apoptosis of the affected cells.

Experiment on emulsion control group revealed that K562 cell proliferation cycle was not notably affected by emulsion with a concentration below 10μl/ml (See Fig 1), which demonstrated by comparison that KLT could significantly influence cancer cell proliferation cycle. Some Japanese scholars reported that they had isolated two components with inhibiting action on Ehrlich ascites carcinoma in mice from the ethanol extract of Coix Seed. One could cause degeneration of cytoplasm and the other could make caryocinesis stagnate at the metaphase of cell proliferation cycle. This study has revealed that KLT might inhibit the proliferation of cancer cells by retarding the mitosis of tumor cells.

References

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Table 1. Effect of KLT injection on K562 Cell Proliferation Cycle

Groups	Concentration	G_1%	S%	G_2+M%
Control		42.4	51.6	6.0
Emulsion	1μl/ml	42	47.4	5.4
	5μl/ml	45.3	52.7	2.0
	10μl/ml	41.0	48.3	10.6
KLT	1μl/ml	26.2	58.1	15.7
	5μl/ml	27.2	24.1	48.8
	10μl/ml	26.4	6.1	67.5
	50μl/ml	-	Death of cells	