The Effects of Kanglaite Injection on Radiosusceptibility of Renal Carcinoma Cells

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Kanglaite Injection (KLT) is an anti-tumor agent prepared from Semen Coicis, a traditional Chinese medicinal herb. Pharmacodynamic and clinical researches of KLT have shown that it has definite therapeutic action of inhibiting the growth of many tumors. Studies have also revealed that KLT is effective in influencing cancer cell proliferation cycle by increasing the ratio of radiosusceptible G2+M phase cells and decreasing the ratio of radio resistant S phase cells.

In this study, we investigated the effects of Kanglaite Injection on increasing cancer cell radiosusceptible in order to achieve better therapeutic effects in cancer management.

1. Materials and Methods
1.1 Drugs and Cell Culture
Kanglaite Injection (10%) and emulsion for control were supplied by Zhejiang Kanglaite Pharmaceutical Co. Ltd.

Granulocyte renal carcinoma cell line (GRC-1) was supplied by the Institute of Urinary Surgery, the First Hospital affiliated with Beijing Medical University. The cells were cultured and passaged in RPMI-1640 culture medium containing 15% calf serum at 37°C, 5% CO₂ in an incubator.

In Situ Cell Death Detection Kits (TUNEL) were purchased from Sigma, U.S.A.

Flow cytometric scan (FAC Scan) was purchased from Becton Dickinson, U.S.A.

1.2 Groups for Radiosusceptibility Experiment
The experiment was performed in a Kanglaite treated group and an emulsion control group. KLT 10μl/ml was added when a single-layer of cells almost formed. The cells were cultured for another 24 hours, digested with trypsin to prepare cell suspension. The cells were split into 2ml culture flasks and irradiated by different irradiation dosage.

The irradiation was performed by a 10 MV-X-ray linear accelerator (Varian 1800, USA) at the dose rate 300cGy/minute. 2cm thick plastic plate was placed on top of the culture flasks. The cells were irradiated at 0, 250, 500, 750 and 1000 cGy. After irradiation, the cells were inoculated according to irradiation dosage gradient into the wells of a 24-well culture plate. The cells were cultured for 8 days, fixed with methanol. Giemsa staining method was
applied. 50 cell colonies were selected. Analysis was performed on computer on a multitarget model.

1.3 Assay of Cell Apoptosis with Terminal Deoxynucleotidyl Transferase (TUNEL) Method

APO-Direct Kit was used. 1×10⁶/ml cells were washed twice with phosphate buffer solution (PBS), fixed with 5% paraformaldehyde for 15 minutes and washed with PBS again. To the cell sediment, 50μl TUNEL labeling mixture was added. The cells were kept at 37°C for 1 hour, washed with PBS, added with Propidium Iodide (PI) 20μl and kept at room temperature for 30 minutes for assay.

1.4 Statistic Analysis

T-test.

2. Results

2.1 The Effects of Kanglaite Injection on Radiosusceptibility of Renal Carcinoma Cells

24 hours after GRC-1 cells were treated by KLT at the concentration of 10μl/ml, the cells were irradiated by different irradiation dosage. It was observed that Do for KLT treated group was 90.44 cGy and Do for emulsion group was 139.40cGy. Statistic analysis revealed that KLT could effectively increase radiosusceptibility of renal carcinoma cells (P<0.05). Sensitivity Enhancement (SE) was 1.54. See Figure 1.

2.2 Assay of KLT Induced Apoptosis of Renal Carcinoma Cells

After renal carcinoma cells were treated with KLT at the concentrations of 0μl/ml, 5μl/ml, 10μl/ml, 15μl/ml and 20μl/ml for 24 hours, the cells were collected. Apoptosis of cells were assayed with APO-Direct Kit. It was observed that the number of apoptotic cells increased when they were treated with KLT of higher concentrations until 10μl/ml. The number of apoptotic cells decreased when they were treated with KLT of increased concentrations higher than 10μl/ml.

3. Discussion

Renal carcinoma cells are not radiosusceptible. Due to the high expression rate of multiple drug resistant gene in renal carcinoma cells (in about 87% cases) the effect of chemotherapy is neither satisfactory. How to improve the local control for renal carcinoma is still a challenge in cancer management.

Apoptosis and necrosis are two different types of death for cells. Necrosis happens when external injury factors act directly on cells, tumefying the cytoplasm and destroying the biomembrane of cells. Apoptosis is an independent process of cell suicide. Cell apoptotic process is manifested by its morphological characteristic-the formation of apoptotic body and biochemical characteristic-the degradation of DNA chains into 180-200bp segments. Gel electrophoretic analysis reveals a characteristic ladder appearance.
Basic studies of Kanglaite Injection have revealed that KLT has the action of retarding tumor cell proliferation cycle at the G₂+M phase and decreasing the percentage of S phase cells. Tumor cells in G₂+M phase are radio susceptible while cells in S phase are unsusceptible to radiotherapy.

In this study, we treated the radio-unsusceptible renal carcinoma cells by KLT 10μl/ml and then irradiate the cells with different irradiation dosage. The study demonstrates that KLT is effective in increasing radio susceptibility of renal carcinoma cells with a SE of 1.54. The analysis of cell apoptosis reveals that KLT, combined with irradiation, has the action of inducing apoptosis of renal carcinoma cells. The optimized KLT concentration for inducing tumor cell apoptosis is 10μl/ml. When the cells were treated by KLT at a concentration higher than 10μl/ml, the percentage of apoptotic cells decreased as a result of the increase of the percentage of necrosis cells. In our another investigation, analysis of the effect of different X-ray irradiation dosage on the ability of renal carcinoma cells to form colonies reveals that an irradiation dosage below 1500 cGy mainly suppresses the ability of renal carcinoma cells to form colonies and that an irradiation dosage of 2000 cGy will induce apoptosis of certain tumor cells. This study demonstrates that after renal carcinoma cells were treated by Kanglaite Injection, KLT not only has the action of inducing apoptosis of the cells, but also is effective in decreasing the number of renal carcinoma cells in radioresistant subgroup and increasing the number of renal carcinoma cells in radio susceptible subgroup and causing reproduction termination of renal cancer cells.

This study reveals that KLT is effective in increasing radiosusceptibility of cancer cells through the mechanism of inducing apoptosis of tumor cells at certain concentration and causing necrosis of tumor cells at higher concentrations. The anti-tumor effects of KLT combined with radiotherapy depend on the concentration of KLT. A dose-effect relationship is present for KLT to induce apoptosis and necrosis of tumor cells.

References