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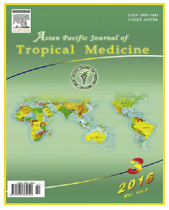

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Mechanism of all-transretinoic acid increasing retinoblastoma sensitivity to vincristine

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ABSTRACT

Objective: To explore the mechanism of all-transretinoic acid (ATRA) increasing retinoblastoma (RB) sensitivity to vincristine, and the inhibiting effect of vincristine combined with ATRA treatment on the SO-RB50 cell proliferation.

Methods: SO-RB50 cells were cultivated by routine culture method. Different concentrations of vincristine or ATRA were added into culture solution. After 48 h, cell counting kit-8 was used to detect the median inhibitory concentration (IC_{50}) of vincristine combined with ATRT treatment to SO-RB50 cells. SO-RB50 cells were divided into drug combination group, vincristine group, ATRA group and control group. Different drugs were added into the culture solution respectively for cell culture based on the IC_{50} value. Cell counting kit-8 was used to detect the cell proliferation every 24-h cultivation. After continuous determination for 6 d, data was processed to draw the cell growth curve. After drug use for 72 h, flow cytometry was used to detect the proportion of different cell cycles of SO-RB50 cells in each group. After drug use for 48 h, annexin V/propidium iodide method was used to detect the SO-RB50 cell apoptosis in each group.

Results: The IC_{50} value of vincristine treatment on the SO-RB50 cells was 0.11 μmol/L, and ATRT was 12.84 μmol/L. The cell growth curve in control group rose gradually along with the extended culture time, but after vincristine and ATRA treatment, the cell growth curve was smooth and steady. The cell increment was the least in drug combination group and its cell growth curve was the smoothest. There was significant difference in A_{450} 48 h and 72 h after treatment ($F_{grouping} = 77.316$, $P < 0.001$; $F_{time} = 86.985$, $P < 0.001$). Compared with control group, A_{450} value in drug combination group, vincristine group, ATRA group was significant lower ($P < 0.001$). Compared with control group, the G_2/M phase cell proportion in vincristine group was significantly increased, while the G_0/G_1 phase cell proportion was significantly decreased; the G_0/G_1 phase cell proportion in ATRA group was significantly increased, while the S phase cell proportion was significantly decreased ($F_{G0/G1} = 85.878$, $F_s = 56.455$, $F_{G2/M} = 85.878$, $P < 0.001$). After 48 h, there was significant difference in SO-RB50 cell apoptosis rate among groups ($F = 11.312$, $P < 0.05$). The apoptosis rate in drug combination group was significantly higher than that of other groups ($P < 0.001$).

Conclusions: ATRA can increase the sensitivity of SO-RB50 cells to vincristine. Vincristine combined with ATRA treatment can significantly increase the inhibiting effect on SO-RB50 cells, which may be related with promoting cell apoptosis and involving in cell cycle control.

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1. Introduction

Retinoblastoma (RB) is a kind of intraocular malignant tumor. Most infants are vulnerable to RB, accounting for 3%–4% of children's malignant tumors. RB has brought a great threat to the eyesight and life of children [1]. Survival rate of children with RB in developed countries is up to 95%, while it is only 50% worldwide [2,3]. Clinical treatments for RB mainly include chemotherapy, radiotherapy, surgery and other local treatments [4]. Among them, chemotherapy plays a very important therapeutic effect. However, the drug resistance of tumor cells easily leads to the chemotherapy failure [5,6]. Therefore, the research hotspot in this world is focused on looking for a kind of more sensitive chemotherapy [7]. All-transretinoic acid (ATRA) can effectively promote the cell differentiation and obviously inhibit the cell proliferation [8]. There are researches showing that ATRA can effectively increase the sensitivity of multiple tumor cells like breast cancer and liver cancer to chemotherapies. However, researches on increasing the sensitivity of RB chemotherapies are relatively less [9,10]. In this study, the mechanism that ATRA could increase the sensitivity of RB to vincristine and the inhibiting effect of vincristine combined with ATRA treatment on the SO-RB50 cell proliferation have been discussed.

2. Meterials and methods

2.1. Materials

2.1.1. Cell sources

SO-RB50 cell lines were provided by Pathology Lab of Zhongshan Eye Center, Zhongshan University. RPMI/1640 culture solution (containing 1% mass fraction of mycillin antibody and 10% volume fraction of fetal calf serum) was used to culture these cells. Culture condition: temperature (37 °C), saturation humidity and volume fraction (5% CO_2). Cells showed a suspended growth and cells in logarithmic phase were selected for detection.

2.1.2. Key instruments and reagents

CO_2 incubator was from Japan SANYO Co.; ELIASA was from Beijing Perlong New counting Co., Ltd.; flow cytometry (FCM) was from America BD Co. RPMI/1640 culture medium and fetal calf serum were from America Hyclone Co.; ATRA was from America Sigma Co.; vincristine was from Zhejiang Hisun Pharmaceutical Company Ltd.; cell apoptosis detection kit was from Beijing 4A Biotech Co., Ltd.; cell counting kit-8 (CCK-8) was from Japan Dojindo Co.; cell cycle detection kit was from Hangzhou MultiSciences Biotech Co., Ltd.

2.2. Methods

2.2.1. IC_{50} value of SO-RB50 cells after vincristine and ATRA treatment detected by CCK-8

SO-RB50 cells in logarithmic phase were inoculated into 96-well culture plates evenly, with 5000/hole. Different mass concentrations of 0.005, 0.010, 0.050, 0.100, 0.500 and 1.000 μmol/L of vincristine and 2.50, 5.00, 10.00, 20.00 and 40.00 μmol/L of ATRA were added respectively. A total of 5 holes were set up for each concentration. After being cultured for 48 h, 10 mL of CCK-8 reagent was added into each hole for 2 h incubation at 37 °C. ELIASA method was used to detect the A_{450} value at 450 nm [11].

2.2.2. Cell proliferation detected by CCK-8

The cultured cells were divided into drug combination group, vincristine group, ATRA group and control group. SO-RB50 cells in logarithmic phase were inoculated into 96-well culture plates, with 2.5×10^4/mL of density. A total of 200 μL was added into each hole. IC_{450} of vincristine (0.11 μg/mL) or ATRA (12.84 μmol/L) was added respectively according to the grouping. Five holes were set up in each group. CCK-8 was used to detect the A_{450} value of cells in each group at 450 nm every 24 h for 6 continuous days. Then the average value was calculated and the cell growth curve was drew [12].

2.2.3. Cell cycle detected by FCM

SO-RB50 cells in logarithmic phase were inoculated into six-well culture plates, with 2.5×10^4/mL of density. Each hole was inoculated with 2 mL. IC_{450} of vincristine or ATRA was added respectively. A total of 72 h after drug treatment, PBS was used for washing for 2 times, with centrifugation for 5 min (1000 r/min and 12 cm of centrifugal radius). A total of 1×10^6 cells were collected in each group and were fixed with ethyl alcohol with 70% of volume fraction. Temperature was controlled at 4 °C and treatment was performed after overnight. After PBS washing for once, 100 μL of buffer solution (containing 100 μg/mL of RNA enzyme and 0.2% volume fraction of Triton X-100) was added for 30 min incubation at 37 °C; then 400 μL of propidium iodide (PI) staining solution was added for 30 min incubation at 4 °C, with keeping out of the sun. FCM was performed to detect the cells of different cell cycles. Cell proportion was calculated [13].

2.2.4. Apoptosis rate of cells detected by annexin V/PI

SO-RB50 cells in logarithmic phase were inoculated into six-well culture plates, with 2.5×10^4/mL of density. Each hole was inoculated with 2 mL. IC_{450} of vincristine or ATRA was added respectively. A total of 72 h after drug treatment, PBS was used for washing for 2 times, with centrifugation for 5 min (1000 r/min). A total of 1×10^6 cells were collected in each group. A total of 100 μL of binding buffer solution was added for cell resuspension. Then 10 μL of annexin V-FITC and 5 μL of PI were added into culture solution for mixing, with incubation for 30 min at 4 °C. Afterwards, incubation for 15 min was performed with keeping out of the sun and 400 μL of binding buffer solution was added once more. FCM was used to make a comparison of the ratio of apoptotic cells [14].

2.3. Statistical method

SPSS 13.0 statistical package was used for experimental data analysis. The data information in this study showed a normal distribution by Shapiro–Wilk test. Data was expressed by mean ± SD and was detected by Levene homogeneity test of variances. Grouping was based on the balanced grouping and multilevel experimental design method. Block design two-way analysis of variance (ANOVA) was adopted to compare the overall difference of A_{450} value changes in different time in drug combination group, vincristine group, ATRA group and control group. *SNK-q* test was used for multiple comparisons among