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Study on the influence of curcumin on chemosensitivity of nephroblastoma cells

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ABSTRACT

Objective: To study the influence of curcumin on chemosensitivity of nephroblastoma cells.

Methods: Human nephroblastoma cells line SK-NEP-1 was transplanted to the nude mice subcutaneously to establish the implantation tumor model of human nephroblastoma cells. A total of 30 tumor-bearing mice were divided into three groups of ten randomly. The routine chemotherapy group was given vincristine (0.05 mg/mL·0.2 mL/d) and actinomycin D (15 ng/mL·0.2 mL/d) combined chemotherapy regime. The curcumin chemotherapy group was given the same combined chemotherapy regimens and curcumin (30 mg/kg/d) by intraperitoneal injection. The control group was given normal saline (NS) of the same volume by intraperitoneal injection. Continuous administration would be kept for 4 weeks and 3 days a week. The volumetric changes of every group were recorded. The serum of every group in different time was collected and the VEGF content was detected by ELISA. All mice were cercrificed and the tumor tissues were stripped and weighed after 4 weeks' treatment. The tumor inhibition rate was calculated. The cell proliferation activity and apoptosis rate were detected by MTT and flow cytometry method. All data were statistically analyzed by SPSS 19.0.

Results: The tumor volume, serum VEGF content, tumor inhibition rate, cell proliferation activity and apoptosis rate of routine chemotherapy group and curcumin chemotherapy group had significant differences comparing with the control group ($P < 0.05$) after 4-week's treatment. The cancer growth of curcumin chemotherapy group was obviously decreased and even tended to shrink comparing with routine chemotherapy group ($\chi^2 = 15.732$, $P = 0.007$). The cell proliferation activity was significantly reduced and the apoptosis rate was significantly higher, ($\chi^2 = 9.427$, $P = 0.012$) which showing the effect of chemotherapy was enhanced.

Conclusions: The chemosensitivity of nephroblastoma cells could be improved by curcumin, then the effect of preoperative adjuvant chemotherapy scheme would be enhanced, the growth of nephroblastoma cells would be inhibited and the surgical risk of nephroblastoma would be reduced.

1. Introduction

Nephroblastoma is one of the most common malignant tumors in children. The incidence of this tumor is only second to neuroblastoma. Pathogenesis is generally considered to be chromosomal deletions or familial inheritance [1], resulting in the differentiation disorder and malignant proliferation of blastemal cells. Thus, the age of onset is generally less than 5 years old, a few are less than 10 years old [2]. In the early stage of the disease, the survival rate is low due to the overall backwardness of tumor clinical research. In recent years, compared with the cure rate of malignant tumors in adults, related researches have improved the cure rate of this disease significantly. Modern clinical treatment measures are

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radiochemotherapy and surgical comprehensive treatment. Five-year's survival rate is generally above 80%. Studies have shown that preoperative adjuvant chemotherapy could effectively reduce the surgical risk of nephroblastoma and improve the prognostic effects [3]. Therefore, preoperative chemotherapy has a pivotal position in the whole process of treatment. Currently, limited drugs used for children's tumor chemotherapy are mainly vincristine, actinomycin D, adriamycin Amycin *etc*. [4,5]. Chemotherapy adapts one or more drugs according to tumor stage, and the effect are different because of children's individual factors. Therefore, the study of nephroblastoma aims to explore a new and effective adjuvant chemotherapy scheme, and improve the effect of preoperative chemotherapy and chemosensitivity.

Curcumin, extracted from curcuma, is an important component of Chinese medicine with the effect of softening heart and cerebral vessels, antioxidation, antisepsis, anti-inflammation and regulating the digestive system [6]. Studies have shown that this compound can inhibit the malignant proliferation of tumor cells [7,8], and enhance chemosensitivity of liver cancer, gastric cancer and colon cancer etc. [9,10], but the research applied to pediatric tumor is rare. This study provides theoretical and experimental basis for the study of new adjuvant chemotherapy, by establishing the implantation tumor model of nephroblastoma cells and implementing different chemotherapy schemes to research the influence of curcumin on chemosensitivity of nephroblastoma cells.

2. Materials and methods

2.1. Materials

Nude mice used in the experiment were purchased from Shanghai Experimental Animal Center, Chinese Academy of Sciences. A total of 36 mice which were 4–5 weeks old were used for this study. Half of then were male mice and half were female mice. The study was performed after the mice were allowed to acclimate for 1 week at Animal Experimental Center of Zhengzhou University. Laboratory was kept sterilized with constant temperature and humidity.

Human nephroblastoma cell line SK-NEP-1 was purchased from the Shanghai Cell Bank, Chinese Academy of Sciences. Curcumin was purchased from Sigma Company. RPMI-1640 and fetal bovine serum were purchased from GIBCIO Company. Rat VEGF ELISA kit was purchased from Santa Cruz Company; DMSO and MTT were purchased from Sigma Company. FITC, and PI were purchased from BD (Shanghai) Co., ltd. Among them, curcumin was dissolved in 10% DMSO- normal saline before 30 mg/kg clear liquid was formulated.

2.2. Methods

2.2.1. Implantation tumor model

The cell cryopreserved tubes were placed in the 37 °C water bath and shaken for thawing out. The cell suspension was transferred to culture bottles with suction tubes and then cultured in RPMI-1640 medium after adding 10% fetal bovine serum and 100 U/mL penicillin and streptomycin. Cells were digested by trypsin every other day and the culture medium was replaced to log phase.

The cell concentration was adjusted to 1×10^7/mL. Before the experiment, right forelimbs of mice were disinfected by iodine, and injected with 0.1 mL cell suspension in 1 mL sterile syringes. After implantation, mice were under close observation for 30 min, and were put back to the cage without adverse reaction.

2.2.2. Treatment in each group

After 7–10 days, 30 mice implanted tumor models were divided into 3 groups. They are routine chemotherapy group, curcumin chemotherapy group and control group respectively. The routine chemotherapy group was intraperitoneally injected with vincristine (0.05 mg/mL·0.2 mL/d) and actinomycin D (15 ng/mL·0.2 mL/d). Continuous administration was 3 days a week and kept for 4 weeks. The curcumin chemotherapy group was given the same combined chemotherapy regimens and curcumin (30 mg/kg/d) by intraperitoneal injection. Continuous administration was 3 days a week and kept for 4 weeks. The control group was given normal saline (NS) of the same volume by intraperitoneal injection. After treatment, tumor's long (a) and short diameters (b) were measured every 3 days, and the tumor volume was calculated with the formula $V = 0.5 \times ab^2$.

2.2.3. Detection of the VEGF content

two mice were randomly selected 1day before treatment and 7 d, 14 d, 21 d and 28 d after treatment, and 2 mL peripheral blood were drew by femoral artery blood sampling method. The serum was obtained after 3 000 rpm centrifugation, and the VEGF protein content in serum samples was detected by ELISA. The specific operating steps were carried out according to the instructions of kit.

2.2.4. Cells proliferation activity

After 4 weeks' chemotherapy, all mice were sacrificed with cervical dislocation and the tumor tissues were stripped and weighed immediately. The tumor inhibition rate was calculated as:

Tumor inhibition rate = (tumor weight of control group − tumor weight of experimental group)/tumor weight of control group × 100%.

Cells suspension was made from tumor specimens and cultured in DMEM medium containing 20% fetal bovine serum and 1% of penicillin and streptomycin. After 48 h, 20 uL MTT was added to absorb culture medium. Four hours' later, the supernatant was discarded after centrifugation. 150 uL DMSO was added to terminate the reaction. The absorbance at 490 nm was detected by ELIASA and the cell activity was detected comparing with the absorbance of control hole.

Cell relative proliferation activity (%) = absorbance of experimental group/absorbance of control group × 100%.