Effect of levofloxacononone chalcone derivatives on the apoptosis and autophagy of HCC SMMC-7721 cells

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Abstract: The aim of present research work is study the effect of levofloxacononone chalcone derivatives on apoptosis and autophagy of HCC SMMC-7721 cells in patients with hepatocellular carcinoma (HCC). The HCC SMMC- 7721 cells in the logarithmic phase growth were inoculated, and the proliferation of SMMC - 7721 cells was detected by MTT assay. The effect of levofloxacononone chalcone derivatives on SMMC- 7721 cell cycle and apoptosis rate was detected by flow cytometry. Cells were treated by autophagy inhibitor chloroquine to validate the effect of levofloxacononone chalcone derivatives on cell proliferation and apoptosis. Levofloxacononone chalcone derivatives could significantly inhibit the proliferation of SMMC-7721 cells. Compared with the control group, the apoptosis rate of cells treated with levofloxacononone chalcone derivatives was increased significantly in 24h, showing significant difference between groups. Chloroquine could increase the inhibitory effect of low-dose levofloxacononone chalcone derivatives on SMMC-7721 cell proliferation, and decrease the inhibitory effect of high-dose levofloxacononone chalcone derivatives on SMMC-7721 cell proliferation. Levofloxacononone chalcone derivatives can obviously induce the apoptosis and autophagy of SMMC -7721 cells, at a low dose, its autophagy can protect cells; and at a high dose, the autophagy can decrease the cell proliferation rate, to promote cell apoptosis.

Keywords: Levofloxacononone chalcone derivatives, HCC SMMC-7721 cell, apoptosis and autophagy.

INTRODUCTION

Human hepatocellular carcinoma is a common, severe, frequently-occurring malignant tumor, with high incidence and shows a gradual upward trend. It is ranked among the top in the incidence of malignant tumors in Europe, United States and other developed countries (Zheng *et al.,* 2012). Most patients were found metastases when diagnosed, with high mortality and it has serious impact on the health and quality of life of patients (Tang *et al.,* 2017). Most researchers believe that the disease occurrence and progression is affected by multiple factors, so that the cancer recurrence is a result of multi-stage, multi-factor, multi-gene and long-term progression (Paccez *et al.,* 2014). Since the primary liver cancer has high drug resistance, with the background of liver disease and toxicity, etc., the systematic chemotherapy of liver cancer has not achieved obvious effect and there are no recognized standard drugs or treatment regimens so far. One of the most crucial factors of poor prognosis in HCC patients is the lack of effective treatment regimens. Most of the chemotherapy drugs have used in clinical treatment, but their therapeutic effect is poor. Generally, the effective rate of single medication will be less than 10%, with obvious toxicity (Shi *et al*., 2012; Ye *et al*., 2015). Therefore, the screening of potentially effective compounds is still one of the important tasks for the treatment of HCC.

Fluor quinolones are used as a class of chemotherapeutic antimicrobials that can be used extensively in clinical practice. Their target site is bacterial DNA gyrase, which is homologous to the primary structure sequence of the topoisomerase in mammalian cells. After transformation, the antibacterial activity sequence of fluoroquinolone is converted to anti-tumor activity, and its effective part of acylhydrazone compound has a strong consistency with the topoisomerase II, which further inhibits the DNA re-link reaction (Sun *et al.*, 2013). Therefore, in this study, we investigated the effect of levofloxacononone chalcone derivatives on apoptosis and autophagy of HCC SMMC-7721 cells, to provide guidance on its clinical treatment.

MATERIALS AND METHODS

General data

Patients were confirmed to suffer from hepatocellular carcinoma according to clinical data. All patients included diagnostic standard and their were pathologically confirmed by the Pathology Department. Human hepatoma cell HCC SMMC-7721 cell lines were used. All projects were approved by the ethics committee of the hospital, signed with informed consent.

Effect of levofloxacononone chalcone derivatives on proliferation of SMMC-7721 cells

HCC SMMC-7721 cells grew in 10% fetal bovine serum medium (volume fraction) and cultured in a saturated humidity constant temperature incubator (37^{o}C, 5% carbon dioxide (CO2)); and the growth conditions were observed every day (Ge *et al.,*2014). When cells grew to the logarithmic growth phase and sub-cultured to a

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sufficient number, one tube was frozen to prepare the experiment. When MTT method was used, HCC SMMC -7721 cells were inoculated in a 96-well plate at a concentration of 1.5 ×107•L^{-1}. The MTT assay is a colorimetric assay for assessing cell metabolic activity. Then the levofloxacononone chalcone derivatives at different concentrations and chloroquine culture medium were added, to culture cells in 48h in the incubator, and then 20μL of 5 g•L^{-1} MTT was added to each well, and incubated for 4 h. The culture medium was drawn out and 150μL of DMSO solvent was added, shaken until the blue crystal was completely dissolved. Then the OD value at 570 nm was measured, to calculate the cell inhibition rate (Li *et al.,* 2015).

Effect of levofloxacononone chalcone derivatives on autophagy of SMMC-7721 cells

After SMMC-7721 cells were treated with 5 μmol•L^{-1} levofloxacononone chalcone derivatives, the total proteins were extracted at low temperature to determine the concentration of protein. Samples were loaded at 20 μg, and appropriate samples were transferred and blocked. The primary antibody was diluted at the concentration of 1/500, shaken on a shaker at 4°C for incubation overnight. The secondary antibody was diluted at the concentration of 1:5×103, then shaken on a shaker at room temperature for 2h for incubation, developed with ECL. The Western blot assay showed that the amount of LC3B-Ⅱ increased with the time of treatment with levofloxacononone chalcone derivatives, compared with the control group, showing significant difference (P<0.01).

Effect of levofloxacononone chalcone derivatives on apoptosis of SMMC-7721 cells

The apoptosis rate was determined by TUNEL method. The HCC SMMC -7721cells were inoculated in a six-well plate with cover glass at the concentration of 1.5 × 107•L-1, 500μL each well (Li *et al.,* 2015). Then it cultured for 24h, detected according to the instructions of kits. The total count of cells per unit area was calculated by Image J software, so as to calculate the apoptosis rate of cells.

STATISTICAL ANALYSIS

The results were analyzed by the SPSS 14.0 statistical software. T-test was used for measurement of data, expressed as mean± standard deviation (###x±s); for count data, X2 test was adopted (expressed by percentage, %). P<0.05 was considered statistically significant difference between groups.

RESULTS

Effect of levofloxacononone chalcone derivatives on proliferation of SMMC-7721 cells

As shown in table 1, levofloxacononone chalcone derivatives alone could significantly inhibit the proliferation of SMMC- 7721 cells in 48h after treatment (P<0.05). Chloroquine could increase the inhibitory effect of low-dose levofloxacononone chalcone derivatives on the proliferation of SMMC-7721 cells, but decrease the inhibitory effect of high-dose levofloxacononone chalcone derivatives on the proliferation of SMMC-7721 cells, showing significant difference between groups (P <0.05).

Effect of levofloxacononone chalcone derivatives on autophagy of SMMC-7721 cells

As shown from the results in table 2, the expression of LC3B-Ⅱ increased with the prolonged time of treatment of levofloxacononone chalcone derivatives, and reached the maximum level at the interval of 6-24 h. Compared with the control group, the expression of LC3B-Ⅱ showed obvious decrease trend in 48 h, with significant difference between groups (P <0.05).

The effect of levofloxacononone chalcone derivatives on apoptosis of SMMC-7721 cells

The number of apoptotic cells increased significantly with the increase of drug concentration, and compared with the control group, there was significant difference in cell numbers (P<0.05). Compared with the same dose of levofloxacononone chalcone derivatives, the apoptosis rate of cells cultured in the 1.25μmol•L-1 levofloxacononone chalcone derivatives combined with chloroquine was higher, while the apoptosis rate of cells cultured in 5 μmol•L-1 levofloxacononone chalcone derivatives combined with chloroquine was significantly decreased, showing significant difference between groups (P <0.05).

DISCUSSION

Hepatocellular carcinoma (HCC) is one of the six common multiple malignant tumors in the world, and it is one of the common causes of deaths of cancers. Patients with HCC have extremely low cure rate, and their 5-year survival rate is low (Zheng *et al.,* 2012). Because of presence of many populations infected with hepatitis B, there is a high incidence of HCC in china, which brings a major threat to people's physical and mental health and life safety. At present, the main treatment method is surgery and chemotherapy- based comprehensive therapy (Paccez *et al.,* 2014). Since the treatment regimen has obvious effect, it is easily restricted by such factors as diagnosis time, liver function and the number of tumors. Also, the presence of blood vessels and lymph node metastases will affect the final effect, causing a low overall prognosis and short survival time of HCC patients (Zhang *et al.,* 2015). It is generally believed that the occurrence and progression of HCC is associated with multi-gene mutation, multi-link and multi-stage evolution (Meng *et al.,* 2013; Paccez *et al.,* 2014). The conduction

Table 1: Inhibitory effect of levofloxacononone chalcone derivatives on proliferation of SMMC-7721 cells

Concentration (μmol·L-1)	Proliferation rate (%)	Proliferation + chloroquine(%)
Control	3.211±0.049	1.0.989±0.0353
0.31	9.095±0.0358*	18.447±0.0281*
0.62	17.096±0.022*	24.084±0.0312*
1.25	20.099±0.087*	29.963±0.055*
2.5	27.077±0.013*	32.077±0.033*
5	32.112±0.0123*	38.081±0.043*

Table 2: The effect of levofloxacononone chalcone derivatives on expression of LC3B in SMMC-7721 cells

h	LC3B-I	LC3B-II
Control	0.192±0.001	0.986±0.013
3	0.196±0.002	0.967±0.011
6	0.979 ± 0.014*	3.050 ± 0.185*
12	0.977 ± 0.013*	2.975 ± 0.076*
24	0.997 ± 0.016*	3. 253 ± 0.146*
48	0.167 ± 0.007*	0.196 ± 0.003*

Table 3: The effect of levofloxacononone chalcone derivatives on apoptosis of SMMC-7721 cells

Concentration /umol·L-1	Apoptosis rate when treated with levofloxacononone chalcone derivatives (%)	Apoptosis rate when treated with levofloxacononone chalcone derivatives+ chloroquine (%)
Control	1.012±0.0006	2.986±0.0013
1.25	27.1960±0.0463*	36.967±0.2113*
5	52.1670 ± 0.0671	43.0862 ± 0.0603*

of cellular signals play an important role during cell growth regulations, since the change of any component in the signaling pathway may affect the regulation of the cell proliferation and apoptosis and induce carcinogenesis (Meng *et al.,* 2013; Dong *et al.,* 2014).

The study results revealed that, compared with the control group, levofloxacononone chalcone derivatives significantly inhibited the proliferation of SMMC-7721 cells. After dosing, the apoptosis rate of cells treated with levofloxacononone chalcone derivatives was increased significantly, showing significant difference between groups. Chloroquine could increase the inhibitory effect of low-dose levofloxacononone chalcone derivatives on SMMC-7721 cell proliferation, and decrease the inhibitory effect of high-dose levofloxacononone chalcone derivatives on SMMC-7721 cell proliferation. Results showed that, levofloxacononone chalcone derivatives can obviously induce the apoptosis and autophagy of SMMC -7721 cells, at a low dose, its autophagy can protect cells; and at a high dose, the autophagy can decrease the cell proliferation rate, to promote cell apoptosis, which can provide guidance for its subsequent clinical treatment.

CONCLUSION

The study results revealed that, compared with the control group, levofloxacononone chalcone derivatives significantly inhibited the proliferation of SMMC-7721 cells. Fluoroquinolones are used as a class of chemotherapeutic antimicrobials that can be used extensively in clinical practice. Their target site is bacterial DNA gyrase, which is homologous to the primary structure sequence of the topoisomerase in mammalian cells. After transformation, the antibacterial activity sequence of fluoroquinolone is converted to anti-tumor activity; and its effective part of acylhydrazone compound has a strong consistency with the topoisomerase II, which further inhibits the DNA re-link reaction.

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