Effect of Curcumin on Apoptosis and Proliferation of Human Hepatocellular Carcinoma WCH-17 Cell Line

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ABSTRACT

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Hepatocellular carcinoma (HCC) is the most common cancer of the liver and the 5th commonest malignancy and also the third leading cause of cancer related death worldwide. Herbal medicines have an important role to prevent cancer. Curcumin is the herbal and dietary spice turmeric with diverse pharmacologic effects including antiproliferative, antiangiogenic and anti-cancer properties. The aim of the present study was to analyze the effect of curcumin on cell growth and apoptosis in the hepatocellular carcinoma WCH-17 cell line. Materials and Methods: MTT assay and flow cytometry assay were used to evaluate proliferative and apoptotic effect of curcumin on WCH-17 cell line. Results: curcumin inhibited the growth of WCH-17 cell and induced apoptosis significantly with a time- and dose-dependent manner. Discussion: Our finding clearly indicated that curcumin has a significant inhibitory and apoptotic effect. Conclusion: curcumin can significantly inhibit the growth of WCH-17 cell and play a significant role in apoptosis.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common cancer of the liver and the 5th commonest malignancy and also the third leading cause of cancer related death worldwide [1]. Mortality rate of the cancer is 600 000 deaths globally per year [2-6]. The most important risk factor for HCC in humans is Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infection [7, 8]. The primary cause of HCC in China and Africa is HBV and in developed countries such as the United States is HCV [8]. Epidemiological study have indicated that a high dietary intake of fruits and vegetables strongly decrease risk of cancer [9-11]. Herbal medicines have an important role to prevent cancer [12, 13]. Curcumin or diferuloylmethane, a polyphenolic molecule extracted from the rhizome of the plant Curcuma longa, is the herbal and dietary spice turmeric and the active ingredient of turmeric with diverse pharmacologic effects including anti-inflammatory, antioxidant, antiproliferative, antiangiogenic and anti-cancer properties. It acts by diverse mechanism such as inhibition of several cell signalling pathways at multiple levels, effects on cellular enzymes and gene transcription by which induces apoptosis [14, 15]. This herbal compound was used as a medicine in many countries over centuries. In this regard, it is important to note that curcumin is a chemopreventive compound with cancer prevention activity by inhibiting specific molecular signaling pathways involved in carcinogenesis without any clinical complication [16-20]. The active ingredient of curcumin is a hydrophobic polyphenol with a characteristic yellow colour, diferuloylmethane.

Curcumin is receiving attention because of its chemopreventive properties against human cancers by which inhibits tumor initiation and induces apoptosis [21-23]. Antitumorigenic activity of curcumin has been reported in certain cancer such as breast, stomach, colon cancers etc. [22-25]. Various in-vitro data have shown that curcumin inhibits proliferation and induces apoptosis in hepatocellular carcinoma J5, Hep G2, Hep 3B and HL-7702 cell lines [26, 27]. Nevertheless, data on the effect of curcumin on hepatocellular carcinoma WCH-17 are rare. Therefore, we decided to evaluate effect of curcumin on viability and apoptosis of hepatocellular carcinoma WCH-17 cell line.

MATERIALS AND METHODS

Human hepatocellular carcinoma cells (WCH-17) were purchased from the National Cell Bank of Iran-Pasteur Institute. Curcumin, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), trypsin-EDTA, fetal...
bovine serum (FBS), Dulbecco’s Modified Eagle Medium (DMEM), streptomycin, penicillin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) was purchased from Merck Co. (Darmstadt, Germany). Curcumin were dissolved in dimethyl sulfoxide (DMSO) and stored in dark at -20°C and then all test concentrations were prepared by diluting the stock solution.

**CELL CULTURE**

The WCH-17 cell line was obtained from the National Cell Bank of Iran-Pasteur Institute. Cells were cultured with DMEM (pH 7.2–7.4) supplemented with 1% sodium pyruvate (sigma), 1.5 g/L sodium bicarbonate, 10% fetal bovine serum and 1% antibiotics including 10,000 units/ml penicillin G sodium (sigma), 10,000 ug/ml streptomycin sulfate and 25 ug/ml amphotericin B (sigma) at 37°C in 5% CO2 to promote attachment. After 24h the cell cultures were incubated with various concentration of curcumin.

**CELL PROLIFERATION ASSAY**

The WCH-17 cells were seeded with 100 μl of complete medium in 96-well plate at a density of 2 × 10^5 cells/well and allowed to adhere and grow for 24 h and then medium was replaced with fresh medium containing different doses of Curcumin (0, 1, 10, 25, 50, 75 and 100 μM) and the cells were cultured for different durations (24 and 48 h). After 24 and 48 h of Curcumin treatment, MTT reagent (5 mg/ml) was added to each well, and incubated for 4 h at 37°C. The formazan crystals were solubilized by the addition of 100 μl of DMSO. The optical density (OD) at 570 nm was measured.

**CELL CYCLE ANALYSIS**

The WCH-17 cells were seeded and grown at a density of 2 × 10^5 cells/well and allowed to adhere and grow for 24 h. After 24 h, the medium was changed and curcumin were added with concentration of 25 μM. After 24, 48 and 72 h of treatment with curcumin, all the adherent cells were collected with 0.05% trypsin and propidium iodide (PI, Becton-Dickinson, San Diego, CA) were used for staining according to the manufacturer’s instructions. The double-stained cells were subsequently analyzed by a FACSCanto flow cytometer (Becton-Dickinson, Mountain View, CA). All experiments were processed independently three times.

**RESULTS**

**EFFECT OF CURCUMIN ON WCH-17 CELL GROWTH**

The effects of curcumin on the growth of human WCH-17 cells in vitro were tested. WCH-17 cells were treated with different concentrations (0, 1, 10, 25, 50, 75 and 100 μM) of curcumin for different time periods (24 and 48 h). To investigate the growth inhibitory effect of curcumin, cell viability was assessed by the MTT assay. As shown in Figure 1, after treatment for 24 and 48 h, a dose-dependent inhibition of growth was observed between 10 and 100 μM of curcumin. The growth of cells decreased to 80, 54, 42, 34 and 30% of the control level after 24h and 72, 50, 38, 30 and 25 % after 48 h with 10, 25, 50, 75 and 100 μM of curcumin respectively (P < 0.001). The IC50 value was 25 μM of curcumin for 24 h.
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Figure 1. Effect of curcumin on WCH-17 cells proliferation. WCH-17 cells were incubated for 24 and 48 h with various concentrations of curcumin. The data are presented as the percentage change compared with the control group. *P<0.001, significantly different from the control group.

EFFECT OF CURCUMIN on the CELL CYCLE

The effect of curcumin on cell cycle progression of WCH-17 cells was investigated by flow cytometry. WCH-17 cells were treated with 25μM of curcumin for 24, 48, and 72 h. Apoptotic cells were determined by flow cytometry using Annexin V/propidium iodide (PI) double labeling. As shown in figure 2, the ratio of the apoptotic cells (Annexin V-FITC-positive) in WCH-17 cells were significantly increased in a time-dependent manner (p<0.001). The percentage of apoptotic cells was reduced following treatment for 24, 48 and 72 h significantly. Maximal apoptosis was observed after 72 h.

Figure 2. Apoptotic effect of curcumin on WCH-17 cells. Data are counted as a ratio of total cells counted. (A) Curcumin induced significant apoptosis in WCH-17 cells in different time periods. (*p<0.001 compared with control).
DISCUSSION

HCC is a common malignancy and is the leading cause of cancer related deaths in the world [28]. Phenolic compounds and related derivatives exhibit inhibitory activities affecting carcinogenesis. A large number of works on the anti-proliferative and anti-carcinogenic effects of these compounds have focused on the evaluation of curcumin. Curcumin is extracted from the plant, Curcuma longa and is well documented that curcumin can induce apoptosis through different mechanisms such as the death receptor mediated pathway and mitochondrial dysfunction [29, 30]. The results of the present study showed that curcumin inhibits proliferation and induces apoptosis in hepatocellular carcinoma WCH-17 cell line. This report is in agreement with those from other studies, showing that curcumin can inhibit proliferation and induce apoptosis in hepatocellular carcinoma Hep G2, Hep 3B and HL-7702 cell lines [26, 27]. Further studies point to the inhibition of cellular migration and invasion of SK-Hep-1 cell line of HCC [31]. In vitro work has shown that curcumin promotes apoptosis in human hepatocellular carcinoma HepG2 cell lines [32]. On the other hand, curcumin increases inhibition of proliferation and apoptosis of SMMC-7721 hepatoma cancer cells through directly or indirectly inhibiting Notch1 expression [33]. Importantly, up-regulation of Notch1 expression has been reported in other cancers such as gastric cancer and esophageal cancer [34-37]. Further, it has been indicated that curcumin can reduce tumor capabilities [38-40]. In another study, it is reported that curcumin suppressed the expression of cyclin D1 to limit cell progression in cell cycle [41]. Other mechanism of curcumin is activation of mitochondria-dependent apoptotic signaling pathway, leading to up-regulation of caspase cascade in canceric cell [42] and also suppressing activation of NF-κB regulated antiapoptotic genes [43]. These findings support our result about apoptotic and antiproliferative effects of curcumin.

CONCLUSION

Our work clearly demonstrated that curcumin can induce apoptosis and inhibit proliferation in human hepatocellular carcinoma WCH-17 cell line.

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REFERENCES