Study on the Mechanism of Paclitaxel Induced Apoptosis of Human Hepatoma Cell Line Hepg2

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Abstract: Objective: To further explore the mechanism of paclitaxel-induced apoptosis of human hepatocellular carcinoma cell line HepG2 based on previous studies. Methods: ①After paclitaxel was applied to HepG2 cells, the colorimetric method was used to determine the changes of SOD activity, MDA content, and GSH content in HepG2 cells. ② The effect of paclitaxel on mitochondrial membrane potential of HepG2 cells was detected by JC-1 staining at different times. Results: ①After HepG2 cells were treated with 5, 10, 20, 40, 80 μg·L-1 paclitaxel for 48 h, the MDA content gradually increased, SOD activity and GSH content gradually decreased, and the concentration was dependent.②JC-1 staining method detected that the mitochondrial membrane potential of HepG2 cells gradually decreased after 24h, 36h, and 48h of 80μg·L-1 paclitaxel on HepG2 cells, and there was a certain time dependence. CONCLUSION: Paclitaxel disrupts the oxidative equilibrium in HepG2 cells, causes oxidative stress in normal metabolic disorders of the cells, thereby oxidatively damages cells, and induces apoptosis in HepG2 cells.

1. Introduction

Liver cancer has a high degree of malignancy, and treatment mainly relies on comprehensive therapies based on surgery. Due to the insidious onset, once it is found that the opportunity for surgery is often lost, the rate of surgical resection is low, and the recurrence rate and metastasis rate are high. Therefore, it is an important and urgent task to seek effective liver cancer treatment with low toxicity and side effects [1]. In previous studies, we confirmed that paclitaxel can induce apoptosis of hepatocellular carcinoma cells HepG2, and the inhibitory effect is concentration-time dependent [2]. Based on previous research, the human hepatocellular carcinoma cell line HepG2 was used as the experimental object to further explore the antitumor activity of paclitaxel, and to explore the effect of paclitaxel on the relationship between the oxidation-reduction balance in HepG2 cells and the cell mitochondrial membrane potential.

2. Material

2.1 Main Reagents and Instruments

D 's Modified Eyre Medium (DMEM) is a product of American Gibco Company. Fetal bovine serum was purchased from Hangzhou Sijiqing Biological Engineering Materials Co., Ltd. Paclitaxel with a purity of 99.7% was purchased from Beijing Huafeng Lianbo Technology Co., Ltd. The SOD detection kit, GSH detection kit, and MDA detection kit were purchased from Nanjing Institute of Biological Engineering, and the BCA protein quantification kit was purchased from Beijing. Biotec Biotechnology Co., Ltd., the inverted phase contrast microscope was purchased from Japan Olympus company, the enzyme-linked immunoassay was purchased from Shenzhen Aikang Biotechnology Co., Ltd.

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3. Methods

3.1 Cell Culture

The human liver cancer cell line HepG2 was cultured in DMEM containing 100 U/ml of penicillin, 50 μg/ml of streptomycin, and heat-inactivated 10% fetal bovine serum. The culture conditions were 37℃, 5%CO2, and saturated humidity. Cells were passaged every 3 to 5 days, and cells in logarithmic growth phase were taken for experiments.

3.2 Preparation of Paclitaxel

Dissolve paclitaxel in dimethyl sulfoxide (DMSO), and then dilute with serum-free DMEM medium to a final concentration of 5, 10, 20, 40, 80 $\mu g \cdot L^{-1}$ for use.

3.3 After Paclitaxel Acts on Hepg2 Cells, Total Protein Concentration is Measured

HepG2 cells were seeded in a 6-well plate and cultured. After adding 5, 10, 20, 40, 80 $\mu g \cdot L^{-1}$ paclitaxel for 24 h, the original solution was removed, washed with PBS twice, and the cells were gently blown down with a pipette. Centrifuge at 1000 rpm for 10 min. Remove the supernatant, resuspend the cells by adding PBS solution, and sonicate the cells in an ice-water bath to make a cell suspension (power: 300w, 5s/times, 30s each time, 4 times in total), and store at -20℃. Prepare the BCA working solution according to the BCA method protein quantification kit. Dilute 10 μL of the standard to 0.5 $mg \cdot mL^{-1}$ with PBS. Add 0, 1, 2, 4, 8, 16, 20 μL of the standard to the protein in the 96-well plate. At the same time, add the appropriate volume of sample to the sample wells of the 96-well plate at the same time, and add 20 μL of PBS solution. Then add 200μLBCA working solution to each well for 30min, measure the absorbance at 595nm with a microplate reader, draw a standard curve and calculate the protein concentration according to the standard curve.

3.4 Colorimetric Determination of the Effect of Paclitaxel on the Activity of Superoxide Dismutase (Sod) in Hepg2 Cells

Prepare the liquid according to the operating procedure of the SOD vitality detection kit, mix the working solution for 10 minutes, and measure the absorbance at 550 nm with a microplate reader. The experiment was repeated 3 times.

SOD activity (U/mgprot) = (A control well-A measurement well)/A control well ÷ 50% × total reaction volume/sampling volume ÷ protein concentration

3.5 Colorimetric Determination of the Effect of Paclitaxel on the Content of Malondialdehyde (Mda) in Hepg2 Cells

Various working solutions were prepared according to the MDA content detection kit, and the absorbance at 532 nm was measured with a microplate reader. The experiment was repeated 3 times.

MDA content (nmol/mgprot) = (A determination-A blank)/(A standard-A blank) × standard concentration ÷ protein concentration of the test sample (mgprot/mL)

3.6 Colorimetric Determination of Paclitaxel on Glutathione (Gsh) Content in Hepg2 Cells

Prepare the liquid according to the operating procedure of the GSH content detection kit, mix the working solution for 5 minutes, and measure the absorbance at 420 nm with a microplate reader. The experiment was repeated 3 times.

GSH content (μM/gprot) = (A determination-A blank)/(A standard-A blank) × standard tube concentration × sample pretreatment dilution multiple ÷ protein homogenate concentration (gprot/L)

3.7 Jc-1 Staining to Detect Mitochondrial Membrane Potential Changes Induced by Paclitaxel in Hepg2 Cells

HepG2 cells were seeded in 6-well plates and cultured with 80 $\mu g \cdot L^{-1}$ paclitaxel for 24 h, 36 h, and 48 h. Remove the culture medium, wash the PBS solution twice, discard the supernatant, add 1 mL of JC-1 working solution and 1 mL of culture medium to each well, and mix thoroughly in a cell

culture incubator at 37℃ and 5% CO2 saturated humidity for 20 min. After discarding the supernatant, each well was washed twice with JC-1 staining buffer, and then 2 mL of culture medium was added and placed under a fluorescence microscope for observation.

3.8 Statistical Processing

The data was processed by SPSS23.0 software. Among them, the measurement data is expressed by $\bar{x} \pm s$, and the count data is expressed by X^2 (%). The measurement data is tested by t. When P <0.05, it indicates that there is difference and the comparison is meaningful.

4. Result

4.1 After Paclitaxel Acts on Hepg2 Cells, Total Protein Concentration is Measured

Take the standard concentration as the abscissa and the absorbance at 595nm as the ordinate to draw the protein standard curve, and derive the standard curve equation as: y = 0.145x + 0.382. The protein concentration of the sample can then be calculated from the standard curve equation and sample absorbance (see Table 1).

Table 1 Protein Concentrations Of Hepg2 Cells with the Treatment of Taxol

Drug concentration($\mu g \cdot L^{-1}$)	Protein concentration($\mu g \cdot \mu L^{-1}$)
0	3.56
5	3.84
10	4.33
20	4.15
40	5.98
80	6.02

4.2 Colorimetric Determination of Sod Activity, Mda Content, and Gsh Content in Hepg2 Cells

HepG2 cells were treated with paclitaxel at 5, 10, 20, 40, and 80 $\mu g \cdot L^{-1}$ for 48 h, and the changes of SOD activity, MDA content, and GSH content in HepG2 cells were detected by colorimetry. (The results are shown in Table 2)

Table 2 Effects of Taxol on the Sod Activity,Mda and Gsh Content of Hepg2 Cells

Group	P /($\mu g \cdot L^{-1}$)	SOD(U/mgprot)	MDA(nmol/mgprot)	GSH(μM/gprot)
Control group	0	82.73±0.31	5.64±0.23	86.15±0.42
Paclitaxel group	5	77.52±0.23	6.10±0.42	83.44±0.37
	10	72.48±0.39	6.38±0.27	80.02±0.19
	20	67.92±0.42	6.72±0.18	75.36±0.51
	40	50.16±0.35	6.81±0.26	73.71±0.35
	80	41.34±0.28	7.25±0.43	64.25±0.22

4.3 Changes of Mitochondrial Membrane Potential Induced by Paclitaxel in Hepg2 Cells

Under a fluorescence microscope, we can observe that the mitochondrial membrane potential of HepG2 cells gradually decreased after a period of 24 h, 36 h, and 48 h of 80 $\mu g \cdot L^{-1}$ paclitaxel, and it was time-dependent.

5. Discuss

Hepatocellular carcinoma is less sensitive to anti-tumor drugs. The heterogeneity and biological characteristics of tumors determine the difference in treatment effectiveness between different individuals. Most studies believe that liver cancer is a drug-resistant disease, and liver cancer is often accompanied by cirrhosis and chronic hepatitis. The tolerability of the drug is also poor. So far, there are few unified and mature drug treatment schemes and fixed treatment models. Finding more effective drugs has important clinical significance [3-4].

Superoxide dismutase (SOD) is an important antioxidant enzyme that can scavenge free radicals

in the body. The measures of aging and death are related to the level of SOD, and may be related to their antagonistic oxygen free radicals [5-7]. Therefore, changes in SOD activity will affect the oxidative balance in the cell. As can be seen from Table 2, after 48 hours of paclitaxel treatment of HepG2 cells, as the concentration of paclitaxel increased, SOD activity gradually decreased. After the cells were treated with paclitaxel at 80 $\mu g \cdot L^{-1}$, the SOD activity decreased to approximately half of the control group and reached 41.06~41.62U/mgprot.

Malondialdehyde (MDA) is a lipid peroxide that can damage the plasma membrane of cells. By measuring its content, we can usually understand the degree of lipid peroxidation in the body, thereby indirectly evaluating the degree of cell damage [8-9]. As can be seen in Table 2, after paclitaxel was applied to HepG2 cells for 48 hours, as the concentration of paclitaxel increased, the intracellular MDA content increased. When the paclitaxel concentration reached 80 $\mu g \cdot L^{-1}$, the MDA content was 6.82 to 7.68 nmol/mgprot.

Glutathione (GSH) is an antioxidant that can participate in the body's redox process, can combine with peroxides and free radicals, and promote cell apoptosis [10-11]. It can be seen from Table 2 that after paclitaxel acts on HepG2 cells for 48 hours, as the paclitaxel concentration increases, the GSH content gradually decreases. After the cells were treated with paclitaxel at 80$\mu g \cdot L^{-1}$, the GSH content decreased to 64.03~64.47μM/gprot.

To sum up, after treating HepG2 cells with 5, 10, 20, 40, 80 $\mu g \cdot L^{-1}$ paclitaxel for 48 h, the MDA content gradually increased, SOD activity and GSH content gradually decreased, and the concentration was dependent. It can be known that paclitaxel disrupts the oxidative equilibrium in HepG2 cells, causes oxidative stress in normal metabolic disorders of the cells, thereby oxidatively damages cells, and induces apoptosis in HepG2 cells.

In addition, the mitochondrial membrane potential of HepG2 cells decreased gradually with the time dependence of HepG2 cells detected by 80$\mu g \cdot L^{-1}$ paclitaxel after 24h, 36h and 48h detection by JC-1 fluorescent probe. It can also be shown that paclitaxel induces apoptosis in HepG2 cells.

Based on previous research, this paper further explores the molecular mechanism of the bioactive natural antitumor drug paclitaxel on HepG2 cells, and provides some reference value for the application of natural antitumor drugs.

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