Original article

Study on apoptosis effect of human breast cancer cell MCF-7 induced by lycorine hydrochloride via death receptor pathway

Yubin Jia,a,b, Miao Yu,a,b,* Zheng Qi,a,b, Di Cuia,b, Guosong Xin,a,b, Bing Wang,a,b, Weiling Jia,a, Lin Chang,a

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ABSTRACT
As research was conducted on the early apoptosis of human breast cancer cell MCF-7 caused by lycorine hydrochloride and the expression of the related apoptosis proteins. The early-period apoptosis rate of human breast cancer cell MCF-7 was tested with the AnnexinV/PI double staining and flow cytometry. The Western Blotting method was also used to detect the protein expression conditions of Fas, Fasl, Caspase-8 and Bid. The results showed that the higher the dose, the higher the rate of apoptosis and that rate of apoptosis was dependent on the dose; the relative protein activity of Fas, Fasl, Caspase-8 and bid gradually rose with the increase of lycorine dosage and the activities revealed certain dose-independence. Results showed that lycorine hydrochloride could induce the apoptosis of human breast cancer cell MCF-7 through the death receptor pathway.

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1. Introduction

Alkaloid is an important chemical component that has a significant pharmacological activity in Chinese herbal medicine and exists widely in nature. Lycorine is a natural alkaloid extracted from Amaryllidaceae. Lycorine and its derivatives have multiple functions such as removing heat, detoxification, expelling phlegm, and alleviating carbunclers, sore throat, edema actions. (Liu et al., 2011) It is particularly significant in the treating tumors (Liu et al., 2016; Iftakhar et al., 2015; Liu, 2012; Evidente et al., 2009).

Death receptor is one of the main pathways that induce cell apoptosis. Eight members of the death receptor family have been characterized so far, which mainly consist of three types of signal transduction pathways, i.e., TNFR, TRAIL and Fas/Fasl signaling pathways. This article will focus on Fas/Fasl pathway. In this paper, we have research on the early apoptosis of human breast cancer cells MCF-7 caused by lycorine hydrochloride and the expression of the related apoptosis proteins, in order to investigate the apoptotic pathways (Chen et al., 2016; Wylli, 2010; Rcuhold and Eschcnhurg, 2012).

2. Material and methods

2.1. Cell

MCF-7 cell line was provided by the Life Science and Environmental Science Research Center of Harbin University of Commerce.

2.2. Reagents and drugs

Lycorine Hydrochloride (mass fraction 98%), Aladdin reagent company; Hydroxy camptothecin (mass fraction 98%), Harbin Medisan Pharmaceutical Co., Ltd.; Fetal bovine serum, Hangzhou Sijiqing Biological Engineering Company; RPMI-1640, American GIBCO company; AnnexinV-FITC apoptosis detection kit, USA BD Biological Technology Co., Ltd.; Goat anti mouse IgG horseradish peroxidase labeling, Beijing Zhongshan Golden Bridge Biotechnology Co., LTD; Mouse anti-actin monoclonal antibody, Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.

2.3. Instruments

DL-CJ-1N type super clean workbench, Beijing donglianha'er Instruments Manufacturing Co., LTD; Adventurer electronic balance, OHAUS Company; CO-150 type carbon dioxide cell incubator,
the United States New Blanche Vick Science Company; CKX-41-32 type inverted microscope, Japan OLYMPUS company; EPICS-XL flow cytometry, the United States Coulter Beckman company; P-type micro displacement device, Gilson company; DYY-7C type electrophoresis instrument, 61 instrument factory; AllegraX-5 low temperature high speed centrifuge, Beckman-Coulter Limited

Fig. 1. The detection of the early apoptosis rate of MCF-7 cells using flow cytometry.
by Share Ltd; JY-SCZ3 type vertical electrophoresis transfer tank, 
61 instrument factory; TL-2000MM-III type multi speed oscillator, 
Jiangyan Tianli Medical Instrument Company; Gel imaging system 
GIS-2019, Tannoon Company.

2.4. Cell culturing and passage

MCF-7 cells were cultured in RPMI-1640 medium supple-
mented with 12% fetal bovine serum and incubated at 37 °C in 
5% CO2 saturated humidity. When the cells reached a high density 
of about 80%, the cells could pass through with the logarithmic 
growth phase of the cells to experiment.

2.5. Apoptosis assays

The MCF-7 cells inoculated in the logarithmic growth phase 
were into a 6-well microtiter plates at 1.0 × 10^5 cells/1-mm-
diameter well. Lycorine solution and HCPT solution was added 
after 24 h. The final concentration of lycorine in the experimental 
group was 3, 6 and 12 μmol/L, the final concentration of HCPT 
group was 2.8 μmol/L, and the control group was added with the 
equal amounts of RPMI-1640 medium. After being cultured for 
48 h, in a 5% CO2 incubator, all the protein of MCF-7 cells incubated for 48 h 
was extracted by centrifugation at 15,000 r/min for 15 min and 
4 °C, the protein content was detected by BCA Protein Concentra-
tion Assay Kit. After 10 min of denaturation at 100 °C for 10 min, 
20 μL of 5 × protein was added to each well, electrophoresed in 
12% SDS - PAGE gel, 80 V to the bottom, and transferred to nitrocel-
lulose membrane. Blots were blocked with 5% non-fat dry milk for 
more than 1 h prior to primary antibody was incubated with at 4 °C 
overnight. After washing the membrane and incubation with sec-
ondary antibodies for 2 h at room temperature, the bands of inter-
est were revealed by DAB and then photographed and analyzed 
with a gel imaging system.

2.6. Protein extraction and western blotting

The MCF-7 cells in the logarithmic growth phase were inocu-
lated into the large culture bottle at 2.5 × 10^5 cells/mL. The final 
concentration of lycorine in the experimental group was 3, 6 and 
12 μmol/L, and the final concentration of HCPT group was 
2.8 μmol/L. The control group was added with the same amount 
of RPMI-1640 medium. After incubation for 48 h at 37 °C in a 5% 
CO2 incubator, all the protein of MCF-7 cells incubated for 48 h 
was extracted by centrifugation at 15,000 r/min for 15 min and 
4 °C, the protein content was detected by BCA Protein Concentra-
tion Assay Kit. After 10 min of denaturation at 100 °C for 10 min, 
20 μL of 5 × protein was added to each well, electrophoresed in 
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overnight. After washing the membrane and incubation with sec-
ondary antibodies for 2 h at room temperature, the bands of inter-
est were revealed by DAB and then photographed and analyzed 
with a gel imaging system.

2.7. Statistical analysis

Statistical analysis was using SPSS 17.0 software. The results 
were expressed as mean ± standard deviation, and t-test used to 
compare the two sample means. There was a statistically signifi-
cant as P < 0.05.

3. Results

3.1. Using Annexin V-FITC/PI double staining to detect early apoptosis 
of cells

The flow cytometry showed that Lycorine acted on human MCF-
7 cells apoptosis induction (Fig. 1). Cells in the first quadrant were 
necrotic and had late apoptotic cells that could be simultaneously 
stained with Annexin V-FITC /PI. The second quadrant cells were 
propidium iodide-negative cells. In the third quadrant, normal

![Fig. 2. Effect of lycorine hydrochloride on the expression of Fas protein in MCF-7 cells.](image2)

![Fig. 3. Effect of lycorine hydrochloride on the expression of Fas protein in MCF-7 cells, compared with the negative control group: *P < 0.05, **P < 0.01.](image3)

![Fig. 4. Effect of lycorine hydrochloride on the expression of FasL protein in MCF-7 cells.](image4)
living cells were not stained with Annexin V-FITC and propidium iodide (PI). The cells in the fourth quadrant were only stained by Annexin V-FITC for early apoptotic cells. The early apoptotic rate of the drug-treated group were (4.67 ± 0.32) %, (7.63 ± 1.10) % and (11.43 ± 1.88) % respectively, and the late apoptotic rate of the positive group was (2.03 ± 0.43) %, which was statistically significant (P < 0.01) between positive group and negative control group. Thus, it could be concluded that as the dose increased, so did the apoptosis rate.

3.2. The detection of the expressions of Fas, FasL, Caspase-8 and Bid by western-blot

3.2.1. The detection of expressions of Fas using western-blot

After the MCF-7 cells were treated with Lycorine (3, 6, 12 μmol/L) for 48 h, the western-blot analysis result showed that the gray value of the Fas protein gradually increased as the dosage increased. The results showed that lycorine could up-regulate the expression of Fas protein. It was statistically significant (P < 0.05) between Fas group and negative control group. The experimental results are shown in Figs. 2 and 3.

3.2.2. The detection of the expressions of FasL by western-blot

After the MCF-7 cells were treated with Lycorine (3, 6, 12 μmol/L) for 48 h, the western-blot analysis result was gray value of FasL protein gradually increased as the dosage increased. The results showed that lycorine could up-regulate the expression of FasL protein. It was statistically significant (P < 0.05) between FasL protein expression and negative control group. The experimental results are shown in Figs. 4 and 5.

3.2.3. The detection of the expressions of Caspase-8 by western-blot

After the MCF-7 cells were treated with Lycorine (3, 6, 12 μmol/L) for 48 h, the western-blot analysis result showed that the gray value of Caspase-8 protein gradually increased as the dosage increased. lycorine could up-regulate Caspase-8 protein expression. It was statistically significant (P < 0.05) between Caspase-8 protein expression and negative control group. The experimental results are shown in Figs. 6 and 7.

3.2.4. The detection of expressions of Bid by western-blot

After the MCF-7 cells were treated with Lycorine (3, 6, 12 μmol/L) for 48 h, the western-blot analysis showed the gray value of BID protein gradually increased as the dosage increased. The results showed that lycorine could up-regulate the expression of Bid protein. It was statistically significant (P < 0.05) between BID group and negative control group. The experimental results are shown in Figs. 8 and 9.
showed that lycorine could up-regulate the expression of BID protein. It was statistically significant (P < 0.05) between BID protein expression and negative control group. The experimental results are shown in Figs. 8 and 9.

4. Discussion

After treating MCF-7 cells with lycorine, and Annexin V-FITC/PI kit test results demonstrated that lycorine could inhibit the growth of MCF-7 cells, and the effect was relatively obvious. The inhibitory rate was dose-dependent, and the inhibition rate increased with the increase of drug dosage (Feng et al., 2016). Thus, it was concluded that lycorine could induce the early apoptosis of the MCF-7 cells.

The western-blot analysis detected the expression of Fas, FasL, Caspase-8 and BID protein, because Fas, Fasl, Caspase-8 and BID proteins are the key proteins in the death receptor pathway of cell apoptotic. The above proteins' expression showed the cells were induced-apoptosis of cancer cells. According to current work findings, lycorine can induce MCF-7 cell apoptosis. The mechanism may be through the up-regulation of Fas/FasL protein expression, thereby promoting the expression of Caspase-8 protein, and activating the downstream Caspase-3, thus inducing a series of related cascaded reactions and triggering apoptosis (Xie et al., 2016; Yine et al., 2015; Lamoral-Theys et al., 2009). It was demonstrated that lycorine could induce MCF-7 cell apoptosis through the death receptor pathway. Many research before promoted the up-regulation of the expression of Bid, it both links the death receptor pathway and mitochondrial pathway, which through the synergistic effect to jointly induce the apoptosis of MCF-7 cells.

5. Conclusions

The results showed that the higher the dosage, the higher the rate of MCF-7 cells’ apoptosis, and the rate of apoptosis was dependent on the dosage; the relative protein activity of Fas, Fasl, Caspase-8 and bid gradually increased with the increase of lycorine dosage, presenting certain dose-independence. Results also showed that lycorine hydrochloride could induce apoptosis of MCF-7 cells through the death receptor pathway.

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