THE EFFECT OF LYCORINE ON THE APOPTOSIS AND MITOCHONDRIAL SIGNAL PATHWAY OF HUMAN BREAST CANCER MCF-7 CELL LINE

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ABSTRACT

Introduction: Lycorine is a natural alkaloid, existing largely in the Amaryllidaceae plant family. It has a wide range of pharmacological activity. Recent studies have found that lycorine has significant effect on the hematological malignancies. However, the effect of lycorine on the Human breast cancer MCF-7 cells (MCF-7) are not clear. In this context, we studied that lycorine induced the apoptosis of MCF-7 cells, and explored the possible mechanism of it.

Materials and methods: The MCF-7 cells were treated with different concentrations of lycorine for 72h. The inhibition rate of the MCF-7 cells was detected with Brominated tetrazolium (MTT) assay, cell morphology was observed using an inverted microscope, the change of cell intracellular calcium and membrane potential was detected with confocal laser scanning microscope.

Results: MTT assay showed that lycorine could inhibit the MCF-7 growth in dose-dependent manner after 72h. The MCF-7 cells were rupture and dead under inverted microscope. Calcium concentration in MCF-7 cells significantly increased and membrane potential were decreased in dose-dependent manner.

Discussion: These results showed that lycorine could inhibit the MCF-7 cells growth and induce it apoptosis, the mechanism might involve an initiation of apoptosis signals in the mitochondrial pathway. This research first discovered anti-tumor mechanism of lycorine on MCF-7 cells.

Keywords: Lycorine, Human Breast Cancer, Apoptosis, mitochondrial pathway.

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Introduction

Cancer is a kind of noncommunicable disease, demonstrating serious threats to human health and life(1). Show according to the data, breast cancer is one of the most common cancer and accounts for 1/3 in women. It is also one of the main causes of death for women(2).

Recent researches have found that the chemical monomers obtained from natural drugs show good results in the treatment of cancer. For example, paclitaxel and camptothecin have a significant therapeutic effect on the cancer, used to treat of ovarian cancer and cervical cancer for many years(3-4).

Lycorine is a chemical monomer isolated from the Amaryllidaceae plant family, existing largely in the bulb(5). With more in-depth study of its pharmacological effects, lycorine is found to have antibacterial, anti-viral, anti-malarial, anti-allergy, inhibition of protein and DNA synthesis, cardiovascular protection and anti-tumor effect(6-8). Meanwhile, lycorine has significant effect on the hematological malignancies and also has cytotoxicity to other human cancer cells(9-10). Up to now, little information is available on the human breast cancer MCF-7 cell apoptosis induced by lycorine.

Apoptosis is initially considered to be a inherent biological phenomenon in cells(11). When the cells are stimulated by certain factors or certain sig-
nals are obtained, apoptosis will occur. It is a gene controlled cell-independent death process, which is an important mechanism to keep homeostasis. This process can effectively remove the cells that do not need. In these conditions, the body will always be in a good state of circulation. It is far-reaching influences to put forward the concept of cell apoptosis for cancer prevention and treatment. At present, there are three ways to induce cell apoptosis: one is to induce apoptosis through the mitochondrial pathway, the second is to induce apoptosis through the death receptor pathway, and the third is to induce apoptosis through the endoplasmic reticulum pathway\(^{(12)}\). Mito-chondria play the most important role in normal cells, as well as the role in the process of cell apoptosis. It is also important to induce cell apoptosis through the mitochondrial pathway.

Recent studies have found that the mechanism is related to a series of apoptosis signal transduction, which can lead to release apoptosis proteins. The mitochondrial membrane potential and \(Ca^{2+}\) assay is a sensitive indicator of mitochondrial function in early apoptosis\(^{(13)}\). Therefore, this paper employed a series of experiments to investigate the cytotoxic activity of lycorine and apoptosis mechanism. Hope to provide a certain theoretical basis for the treatment of breast cancer.

**Materials and methods**

Human breast cancer MCF-7 cells was supported by Research Center on Life Science and Environmental Sciences, Harbin University of Commerce.

Lycorine was extracted and purified from Crinum, Hydroxycamptothecin (HCPT) was obtained from Harbin Shengtai Pharmaceutical Co., Ltd.. RPMI-1640 culture solution, trypsin, fetal bovine serum were purchased from Hangzhou Evergreen Biological Engineering Co., Ltd., Brominated tetrazolium (MTT) and dimethyl sulfoxide (DMSO) were obtained from Tianjin Bodi Chemical Co., Ltd., Tetra nitrogen tetrazolium was supported by Sigma Co., Ltd., Rhodamine 123 and Fluo-3/AM were bought from Beyotime Institute of Biotechnology and Molecular probe Co., Ltd., respectively.

CO-150-type CO2 incubator was supported by Sanyo Electric Co., Ltd.. DL-CJ-Ind type medical clean bench was purchased from East Union Hal Instrument Manufacturing Co., Ltd.. Model 680 microplate reader was purchased from Bio-Rad, USA, CKX-41-32 inverted microscope was purchased from OLYMPUS, Japan, SP2 type laser scanning confocal microscope was purchased from Leica, Germany.

Human breast cancer MCF-7 cells in logarithmic growth phase were digested by 0.25% trypsin for the preparation of cell suspension. Cells were cultured in 96 well plates (5 \(×\) 104 cells/well) overnight prior to the treatment. Then, the cells were treated with lycorine (3, 6, 12 \(\mu\)mol/L), HCPT(1.4, 2.8, 5.6 \(\mu\)mol/L, positive group) and RPMI-1640 medium (control group), 100 \(\mu\)L every well, six parallel holes every group. After72 h, supernatant of the holes was discarded and 100 \(\mu\)L MTT of 0.5 mg/mL in serum-free medium was added in the well. Cultured for 4 h, 200 \(\mu\)L DMSO was added into per hole after discarding supernatant. OD value of each hole under detection wavelength 570 nm was detected by MK3 type microplate reader. The ratio of inhibition of Se-CSPS was calculated according to the following formula and the IC50 were obtained from the ratio of inhibition\(^{(14-18)}\).

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\text{Ratio of inhibition (\%) = (1 - OD value of Se-CSPS group/OD value of control group) ×100.}
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Human breast cancer MCF-7 cells in logarithmic growth phase were digested by 0.25% trypsin for the preparation of cell suspension. Cells were cultured in 96 well plates (5 \(×\) 104 cells/well) overnight prior to the treatment. Then, the cells were treated with lycorine (3, 6, 12 \(\mu\)mol/L), HCPT(2.8 \(\mu\)mol/L, positive group) and RPMI-1640 medium (control group). After 72h, MCF-7 cells observed under inverted microscope.

Human breast cancer MCF-7 cells in logarithmic growth phase were digested by 0.25% trypsin for the preparation of cell suspension. Cells were cultured (1 \(×\) 106 cells/well) overnight prior to the treatment. Then, the cells were treated with lycorine (3, 6, 12 \(\mu\)mol/L), HCPT(2.8 \(\mu\)mol/L, positive group) and RPMI-1640 medium (control group). After 48h, cells were collected by centrifugation (1500r/min, 10min) and were added into Fluo-3/AM (1\(\mu\)g/mL) for 1h, at 37 °C. Then, Fluo-3/AM was discarded by PBS. Finally, 200 \(\mu\)L cell suspension was detected by confocal laser scanning microscope (excitation wavelength: 488nm, emission wavelength: from 540 to 570 nm)\(^{(19-23)}\).

Human breast cancer MCF-7 cells in logarithmic growth phase were digested by 0.25% trypsin for the preparation of cell suspension. Cells were
cultured (1 × 10^4 cells/well) over-night prior to the treatment. Then, the cells were treated with lycorine (3, 6, 12 μmol/L), HCPT (2.8 μmol/L, positive group) and RPMI-1640 medium (control group). After 48h, cells were collected by centrifugation (1500r/min, 10min) and were added into Rhodamine123 (1μg/mL) for 1h, at 37 °C. Then, Rhodamine123 was discarded by PBS. Finally, 200 μL cell suspension was detected by confocal laser scanning microscope (excitation wavelength: 488nm, emission wavelength: from 540 to 570 nm) (24-27).

The data were expressed as means±SEM (standard error of the mean). SPSS 17.0 soft-ware (Statistical Product and Service Solutions) was used to analyze the experimental data. Statistical comparison within groups was carried out by one way ANOVA. A p-value of less than 0.05 was considered to be significant statistically. All determinations were carried out in triplicate.

Results

The inhibitory effect of lycorine on MCF-7 cell proliferation

MTT results were shown in Figure 1. Compared with control group, the inhibition rates of lycorine groups were significantly than that of control group (P<0.05).

![Fig. 1: Inhibition effect of lycorine and HCPT on MCF-7](image)

The OD value in lycorine groups (3, 6, 12 μmol/L) were 0.748 ± 0.060, 0.555 ± 0.060 and 0.460 ± 0.046, re-spectively. The inhibition rates were 40.870%, 56.126% and 63.636%, respective-ly. The OD value in positive group (HCPT 1.4, 2.8, 5.6 μmol/L) were 0.700 ± 0.071, 0.531 ± 0.055 and 0.373 ± 0.017, respectively. The inhibition rates were 44.664%, 58.024% and 70.514%, re-spective-ly. The OD value in control group was 1.265 ± 0.074. It suggested that lycorine could inhibit MCF-7 proliferation in dose-dependent manner (from 1 to 4μmol/L) and IC_{50} of ly-corine was 4.829 μmol/L.

The effect of lycorine on MCF-7 cell morphology

It could be seen from Figure 2 that with the increase of lycorine, there are some changes in cell morphology. MCF-7 cells in control group were intact and showed clear outline. The cell membrane was not broken. MCF-7 cells in positive group (HCPT) showed that part of the cell shape turned round and brust. The density of cell growth was smaller than that of the control cells. MCF-7 cells in lycorine groups gradually appear changes that the surfaces of MCF-7 cells were wrin-kled and the cells were round, showed fragmentation, brust and floated in the cells suspension.

![Fig. 2: Effect of lycorine on MCF-7 cells morphology.](image)

The effect of lycorine on Calcium in MCF-7 cell

Fluorescence intensity of Ca^{2+} was shown in Figure 3, Figure 4. Compared with control group, fluorescence intensity of Ca^{2+} in MCF-7 remarkably increased with the dose increase (P<0.01). Fluorescence intensity of Ca^{2+} in lycorine groups were 19.99 ± 0.07, 25.37 ± 0.22 and 37.66 ± 0.47, respectively. In control group was 14.56 ± 0.15, and in positive group (HCPT) was 20.32 ± 0.07.

![Fig. 3: Effects of lycorine on intracellular fluorescent intensity of Ca^{2+} in MCF-7.](image)
Fluorescence intensity of ΔΨm (mitochondrial membrane potential) was shown in Figure 5, Figure 6. It could be seen that fluorescence intensity of ΔΨm in MCF-7 significantly decreased with the dose increase (Compared with control group, P<0.01). Fluorescence intensity of ΔΨm in lycorine groups were 22.74±0.69, 19.93±0.05 and 18.08±0.89, respectively. In control group was 29.40 ± 0.15, and in positive group (HCPT) was 22.97 ± 0.69.

Discussion

MTT assay is the most effective method to detect the cytotoxicity of drugs to tumor cell. The main principles are as follows. Succinate dehydrogenase in the mitochondria of living cells can restore exogenous MTT to water-insoluble Formazan which is the blue-purple crystals, and deposite in the cell. The dead cells have no such function. DMSO can dissolve the formazan in the cells, and enzyme labeling can detect its absorbance at a specific wave-length(28). This paper explored MTT assay to detect cytotoxicity of lycorine to human breast cancer MCF-7 cells. The result indicated that the lycorine could significantly inhibit the proliferation of MCF-7 cells.

When the cells occur apoptosis, there will be a series of unique morphological characteristics. For example, changes in the volume of cells and gradually become smaller, the chromatin is densely distributed in cells, the chromosome appears broken pieces, the apoptosis body is formed and so on. morphology also could reflect on the cells status. So apoptosis can be judged by the changes of cell morphology(29). The morphology showed that MCF-7 cells were prone to wrinkle and fragment. This implied that lycorine could inhibit the proliferation by apoptosis.

It is found that mitochondrion play an important role in the process of apoptosis, and the mitochondrial pathway is also an important pathway of apoptosis signal transduction. Some studies have confirmed that the changes of intracellular Ca²⁺ concentration and mitochondrial membrane potential are closely related to the mitochondrial pathway(30). The mitochondrial membrane potential and Ca²⁺ assay is a sensitive indicator of mitochondrial function in early apoptosis(13). Lots of organelles in cells contain Ca²⁺, such as mitochondria, endoplasmic net, Golgi apparatus. Especially, mitochondria is the most important Ca²⁺ storage sites. Intracellular Ca²⁺, as a control center in apoptosis signaling pathway, plays an important role. Under a normal state, intracellular Ca²⁺ is in a relatively balanced state. When the cells are stimulated under certain conditions, Ca²⁺ is released. The excessive Ca²⁺ is ingested by the mitochondria, which leads to the damage of the mitochondria and causes a series of changes(31-32).

Mitochondrial membrane potential protect physiological function of mitochondrial, such as the generation of ATP. Mitochondrial membrane potential is closely related to the concentration of Ca²⁺. Mitochondria are cystic structure, surrounded by two membranes. The outside is positively charged, while the interior is negatively charged. Under normal condition, the mitochondrial membrane potential is in a relative equilibrium state. When the Ca²⁺ concentration changes and the mitochondria are damaged, the mitochondrial membrane potential will be lost and this process will be irreversible(33-34).

The mitochondria transmembrane potential (ΔΨm) is the one of best indicators to reflect of the mitochondrial membrane permeability. The un-intact mitochondrial membrane lead to the opening of mitochondrion permeability transition pore and release of cytochrome C, followed by activation of caspase pathway, leading to apoptosis. The experimental results showed that the lycorine could significantly increase Ca²⁺ concentration of human breast cancer MCF-7 cells and decreased mitochondrial membrane potential in a dose-dependent manner.

In summary, lycorine can induce apoptosis in human human breast cancer MCF-7 cells through a mitochondrial pathway. Further research on apoptosis mechanism of lycorine will be carried out in the future.
Conclusion

These studies have shown that lycorine can inhibit the growth of human breast cancer MCF-7 cells and can induce cell apoptosis. The cell apoptosis mechanism of MCF-7 may be related to the increase of intracellular calcium concentration and the decrease of mitochondrial membrane potential caused by lycorine. The emergence of the above situation will damage the function of mitochondria and increase the permeability of mitochondrial membranes. Finally, some of the apoptotic proteins are released and mitochondrial apoptotic pathway is activated.

References


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