

# Study on the Effect of Shikonin on Apoptosis of Breast Cancer Cells

Xi Liu

Qiqihar Medical University, Qiqihar, Heilongjiang, 161006

**Keywords:** Breast Cancer Cells, Apoptosis, Shikonin

**Abstract.** Outside the expert analysis of its chemical composition, has been isolated, including naphthoquinones, fenels, alkaloids, including a variety of compounds and their derivatives. Among them, lipophilic naphthoquinone shikonin has many pharmacological activities, which is the main active ingredient of Lithospermum. Shikonin on breast cancer, liver cancer, prostate cancer and other cancer cells have to kill or inhibit the growth of the role. However, the current mechanism of action of shikonin against breast cancer is not entirely clear.

## Introduction

Breast cancer is a common malignancy in women. Resistance resistance is one of the main causes of poor chemotherapy in breast cancer. Therefore, there is an urgent need to develop new chemotherapeutic drugs to meet the needs of clinical treatment of breast cancer. Lithosin is one of the active ingredients of Lithospermum erythrorhizon. In vitro studies have shown that shikonin can inhibit the growth and proliferation of various tumor cells and induce the apoptosis of tumor cells. In this study, we investigated the inhibitory effect of shikonin on the growth of several breast cancer cell lines (MCF-7, MCF-7 / ADR, MDA-MB-231, SK-BR-3) and the induction of apoptosis , For the treatment of breast cancer for the clinical treatment of breast cancer to provide a theoretical basis.

## Material and Method

Breast cancer cell lines MCF-7, MCF-7 / ADR, SK-BR-3, MDA-MB-231, leukemia cells K 562 provided by Shanghai Fudan Zhangjiang Biomedical Company. (US product of Merck); injection of doxorubicin hydrochloride (10mg each) for the Zhejiang Haizheng Pharmaceutical Co., Ltd. is limited to the United States and the United States, the United States and the United States, The anti-PARPP85, anti $\beta$ -actin, anti-ERK 1/2, HRP-labeled donkey anti-rabbit IgG, HRP-labeled goat anti-mouse IgG and anti-ERK 1/2, HRP-labeled donkey anti-rabbit IgG, HRP-labeled goat anti-mouse IgG and caspase inhibitors (Z-VAD-FMK) are products of Promega USA; HRP Fluorescent Substrate (ECL) is the product of Piercebiotech.

1) Cell culture: MCF-7 and other cells were cultured in DMEM medium (10% extinction of fetal bovine serum and 1% streptomycin), MCF-7 / ADR was added 3 times in medium Adriamycin (final concentration of  $0.4 \mu\text{g} \cdot \text{mL}^{-1}$ ) to maintain the selection pressure, 1 week before the test with no adriamycin DMEM medium.

2) MTT Method: according to the literature, the measurement result using ANOVA statistical analysis methods, the estimation process shikonin effect is calculated using a linear regression method of half maximal inhibitory concentration (an IC<sub>50</sub>), determined using the t test The statistical significance of the inhibitory effect of shikonin on the growth of different cells.

3) Cell morphology observation: Hechst33258 fluorescence staining was mainly carried out according to the literature.

4) SDS-PAGE and immunoprecipitation, ECL chemiluminescence detection: according to the literature.

5) Flow cytometry detection of apoptosis mainly with reference to the literature, the adoption of the u test to determine the effect of shikonin treatment of statistical significance.

The experimental procedure is as follows: 1, cell treatment: no serum dosing, respectively, do two groups, one group with P-JNK inhibitor sp600125 early treatment of cells 1h, and then add lignin (final concentration 0,1,5,10 And 20 $\mu$ Mol / L) for 12 h, 2, protein sample preparation: remove the cell sample aspiration medium placed on ice, washed with PBS buffer once, adding RIPA cell lysate (adding protease inhibitor and phosphorylase Inhibitor) on the ice for 30 min, 12,000 rpm 4 ° C for 10 min. 3, BCA method to determine the protein concentration: ReagentA and Reagent B by 50: 1 ratio of mixing; with a good Reagent mixture added to each hole, each hole 200  $\mu$ L, the standard (0. 1,0. 25 0.05, 1.0 and 2. 0 mg / m L) and 2  $\mu$ L of the test sample were added to the 96-well plate. After 30 min incubation at 37 ° C, the absorbance of OD560 was measured by a microplate reader. And the sample protein concentration was converted according to the standard curve. 4. Preparation of Protein Sample: Mix the protein sample with 4  $\times$  SDS Loading Buffer in 1: 3 ratio and cook for 10 min at 100 ° C. 3000 rpm centrifugation 3 min. 5, SDS-PAGE electrophoresis: 10% of the separation of the use of glue, in accordance with the order of each lane of each treated sample 30  $\mu$ L, two samples on both sides of the sample set aside to add the protein Marker, the extra channel to add 30  $\mu$ L 1  $\times$  SDSLoading Buffer for balance. 80V voltage to the protein Marker separation after the voltage transferred to 120V, Loading Buffer electrophoresis to the bottom of the gel to stop electrophoresis. 6, transfer film: the first PVDF membrane immersed in methanol for 5 min, filter paper, PVDF film, sponge pad into the transfer buffer (4 ° C pre-cooling) in the full soaking. After the electrophoresis is completed, double the glass plate is opened and the transfer device is installed in the following order: black plate (cathode), sponge, filter paper, gel, PVDF film, filter paper, sponge, red plate (anode). Each layer is placed in the need to drain bubbles and the edges are strictly aligned. Install the transfer film device into the electric transfer slot, add the transfer buffer, each film 150 m A constant current for electric rotation. After the transfer film was finished, the PVDF membrane was removed. 7, closed: remove the PVDF membrane 5% skim milk powder room temperature placed in the shaker closed 1 ~ 2 h. 8, the first antibody incubation: according to the required protein size, control Marker position, cut PVDF membrane, add the appropriate primary antibody, 4 ° C shaker incubation overnight. 9, the second antibody incubation: the first resistance after incubation, with tweezers to remove the PVDF membrane, in the TBST buffer repeated rinse 3 times, each 10 min. Add the appropriate secondary antibody (5% skim milk diluted 5000 ~ 10000 times), room temperature incubation 1 ~ 2 h. 10, development: secondary antibody incubation PVDF membrane after the end, with TBST buffer repeated shock rinse three times, each 10 min. Mix the ECL (Pierce) kit A solution, B solution 1: 1 (v / v) and dilute it with dd H<sub>2</sub>O 1: 1, drop on the surface of the PVDF membrane, apply evenly in the dark room with medical X-ray film for several seconds Or longer. Developer development, fixing solution fixing. Western blot analysis was performed using Image J image processing software.

## Results and Analysis

The results showed that shikonin had significant growth inhibitory effect on the four kinds of breast cancer cell lines ( $P < 0.01$ ), and it was time - and dose - dependent. The IC<sub>50</sub> of shikonin on MCF-7, SK-BR-3, MDA-MB-231, MCF-7 / ADR cells were ( $4.79 \pm 0.42$ ), ( $6.79 \pm 0.45$ ), ( $9.27 \pm 0.54$ ), ( $5.23 \pm 0.39$ )  $\mu$ mol  $\cdot$  L<sup>-1</sup>. In view of the low levels of IC<sub>50</sub> of shikonin in several cells in the range of 5 ~ 10 $\mu$ mol  $\cdot$  L<sup>-1</sup>, MCF-7 cell lines were mainly expressed in the following experiments.

The apoptotic degree of MCF-7 increased with the increase of treatment concentration ( $P < 0.01$ ). The cell cycle was arrested in S phase and S phase ( $P < 0.01$ ). The degree of apoptosis was further improved after 48 hours ( $P < 0.01$ ). After 48 h of MCF-7 cells treated with 5  $\mu$ mol  $\cdot$  L<sup>-1</sup>-shikonin, the cell cycle was blocked in the S phase, but 10  $\mu$ mol  $\cdot$  L<sup>-1</sup>-shikonin treated MCF-7 cells for 48 h, The proportion of cells in the cell cycle was significantly reduced, resulting in S phase cells significantly reduced ( $P < 0.01$ ). Shikonin different concentrations of MCF-7 cells treated for 24h, Western blotting detection cell culture lysate supernatant 85kuPARP level of expression, the results show a positive correlation with the strength of shikonin concentration PARP 85ku fragment, the test was repeated three times. In caspase Z-VAD-FMK inhibitor on MCF-7 cells after 1h hormone treated

cells treated with different concentrations of comfrey 85kuPARP detecting the expression level of 24h, visible Z-VAD-FMK inhibited the generation of PARP cleavage fragments, The test was repeated three times.

The expression of 85kuPARP in the supernatant of the cell culture was detected by immunofluorescence. The results showed that the expression of PARP 85ku fragment was significantly higher than that of the control group ( $P < 0.05$ ) Strong and shikonin concentration was positively correlated, test repeated 3 times. In caspase Z-VAD-FMK inhibitor on MCF-7 cells after 1h hormone treated cells treated with different concentrations of comfrey 85kuPARP detecting the expression level of 24h, visible Z-VAD-FMK inhibited the generation of PARP cleavage fragments, The test was repeated three times.

Shikonin is a kind of naphthoquinone compounds extracted from the roots of *Lithospermum erythrorhizon*. In vitro studies have also found that it has the ability to induce apoptosis of various tumor cells. The breast cancer cell lines used in this study have their own characteristics, in which MCF-7 is estrogen receptor-positive (ER +), and high metastatic MDA-MB-231 is ER- and has higher ERK 1 / 2 expression of activity MCF-7 / ADR is adriamycin-induced adriamycin-resistant strains with varying degrees of deletion of ER, SK-BR-3 overexpressing epidermal growth factor receptor (EGFR), has confirmed ER and EGFR Of the high expression and development of breast cancer and prognosis is closely related. This study shows that shikonin has a significant growth inhibitory effect on the above four kinds of breast cancer cell lines, including adriamycin-resistant strain MCF-7 / ADR, and is dependent on drug dose and time. Wu Zhen et al reported that the inhibitory effect of shikonin on the growth of MCF-7 was not obvious, and the results were slightly different from the results of this paper. The analysis may be the difference of culture conditions of MCF-7 cell lines stored in different laboratories. Zhu Yi et al. MCF-7 cells were different in estrogen from different laboratory sources. MCF-7 cells had the longer incubation time in xerogon culture medium, which was more sensitive to ER. Therefore, the growth characteristics of MCF-7 cells in different origins were different. The sensitivity of certain drugs may be different.

Apoptosis is often accompanied by typical morphological and physiological changes in cells, such as increased cell membrane permeability and valgus, DNA breakdown within the nucleus and cell cycle appears sub-diploid peak and so on. Hela cells, colorectal cancer cells CCL229, human tongue squamous cell carcinoma cell line Tca-8113 and the study of breast cancer cell lines, treated with shikonin after the typical apoptosis characteristics. The results of this study show that the cell proliferation of MCF-7 and MCF7-ADR cells treated with shikonin is blocked in S phase, while that of Kupfferin-induced K 526 cells is arrested in G1 phase, and it is reported that shikonin Malignant melanoma cell line A 375-S2, human nasopharyngeal carcinoma cell CNE 2, respectively, the cell cycle stagnation in G1 and S phase, shikonin different cell cycle arrest may be different, the mechanism remains to be further studied. And the sensitivity of S-phase cells to drugs is also high, but also increase the selectivity of drugs, which has a positive effect on the anti-tumor effect of shikonin, chemotherapy drugs induce tumor cell apoptosis and cell cycle , The study found that topoisomerase inhibitors such as VP-16, MTX, CAM and other cells often block the cell cycle in the S phase, the analysis may be due to drug inhibition of topoisomerase after DNA replication blocked, Cell sensitivity is higher.

Caspase protease (cysteine aspartic acid protease) family plays an important role in Fas and TNF-receptor signaling pathways, and is closely related to apoptosis. The currently reported Caspase family has 14 members, of which activated caspase2, 8, 9, 10 are thought to be apoptotic initiators to hydrolyze and activate caspase3, 6, 7, while the latter further cleaves the corresponding substrate, Such as PARP, ICAD and so on. PARP cleavage is thought to be an early molecular marker of apoptosis. The results of this study show that shikonin-induced apoptosis of MGF-7 cells induced PARP lysis, and broad-spectrum caspase inhibitor can better inhibit the parabolysis of PARP, the results of the initial reveal of shikonin-induced apoptosis of breast cancer cells The mechanism of action that breast cancer cell apoptosis may be via the caspase pathway. The results showed that the activity of caspase3, 8 was decreased and the expression of ICAD was decreased and the lysis was induced. The results of Colo-205 treated with shikonin were also found in the

treatment of Hela cells induced by shikonin. Citrin through the activation of caspase3 induced PARP and DF45 degradation to start the target cell apoptosis. The above results have revealed the mechanism of shikonin inducing apoptosis of different kinds of tumor cells in different degrees, which laid a foundation for the clinical treatment of eosinophil for breast cancer and other tumors. In recent years, epidemiological investigation was related to breast cancer at home and abroad show that the incidence continued to rise significantly, chemotherapy plays an important role in the treatment of breast cancer in the whole process, in view of the toxic side effects of chemotherapy in breast cancer treatment and clinical appear Of the resistance to resistance, the urgent need to develop new chemotherapy drugs to meet clinical needs. The results of this study show that shikonin for breast cancer treatment of good expectations, further research still need to combine in vivo and clinical trials to clarify the application prospects of shikonin.

## References

- [1] Deng Shixian, Shi Yuehua. Lithospermate pharmacological research [J]. Yunnan Medicine. 1983 (04)
- [2] Zhang Zhen, Zhang Genhua, Liu Qin, He Xujun. Shikonin induced autophagy inhibition of pancreatic cancer cell proliferation experimental study [J]. Zhejiang Journal of Integrated Traditional and Western Medicine. 2013 (03)
- [3] Hou Ying, Cao Wei, Zhang Xiaonan, Li Xiaoqiang, Liu Shuihong, Li Tao. Inhibitory effect of shikonin on proliferation of human hepatoma HepG2 cells and induced apoptosis [J]. Advances in Modern Biomedical Sciences. 2008 (09)
- [4] Zhao Ruijie, Li Zonggan, Wang Hui, Wang Guangbin, Guan Weijun. Effects of Shikonin on Proliferation and Apoptosis of Hepatoma Cells [J]. Heilongjiang Journal of Animal Husbandry and Veterinary Medicine 2010 (07)
- [5] Rui Zhuanghua, Liang Yongjie. The inhibitory effect of shikonin on the growth of lung adenocarcinoma A549 cells and its mechanism [J]. Journal of Tongji University (Medical Sciences). 2014 (03)
- [6] Qi Shihai, Huang Rongyun. Effects of Shikonin on Proliferation and Melanogenesis of Human Melanoma Cells [J]. Journal of Clinical Pharmacology. 2010 (09)
- [7] Han Jie, Weng Xinchu, Bi Kaishun. In vitro anti-cancer effect of active ingredients in Lithospermum erythrorhizon [J]. Fine Chemicals. 2007 (05)