JTCM 中医杂志 Journal of Traditional Chinese Medicine

Online Submissions: http://www.journaltcm.com
info@journaltcm.com

J Tradit Chin Med 2016 April 15; 36(2): 225-230
ISSN 0255-2922

EXPERIMENTAL STUDY

Inhibitory effect of oridonin on proliferation of RPMI8226 cells and the possible underlying mechanism

Hao Yaning, Zhao Fei, Luo Yuanyuan, Zhang Mei, Li Shasha

Hao Yaning, Zhao Fei, Department of Nephrology, First Affiliated Hospital of Medical School, Xi'an Jiaotong University, Xi'an 710061, China
Luo Yuanyuan, Zhang Mei, Department of Hematology, First Affiliated Hospital of Medical School, Xi'an Jiaotong University, Xi'an 710061, China
Li Shasha, Department of Nephrology, First Affiliated Hospital of Xi'an Medical University, Xi'an 710086, China
Supported by Science and Technology Planning Project of Shaanxi Province (No. 2008K09-09)
Correspondence to: Prof. Hao Yaning, Department of Nephrology, First Affiliated Hospital of Medical School, Xi'an Jiaotong University, Xi'an 710061, China. haoynhaoyn@163.com
Telephone: +86-29-85323953
Accepted: July 15, 2015

Abstract

OBJECTIVE: To observe the effects of oridonin on proliferation and apoptosis of myeloma RPMI8226 cells and to investigate the potential underlying mechanisms.

METHODS: RPMI8226 cells were treated with various concentrations of oridonin. Cell proliferation was analyzed using the thiazolyl blue tetrazolium bromide method. Ultramicrostructure was observed by transmission electron microscopy. Annexin-V/PI staining and flow cytometry was performed to determine cell apoptosis. Expression of apoptosis-related proteins was evaluated by western blot analysis.

RESULTS: Oridonin suppressed the proliferation of RPMI8226 cells and induced apoptosis in a time- and dose-dependent manner. Transmission electron microscopy confirmed apoptotic morphology upon treatment with 20 μmol/L oridonin and western blot revealed decreased expressions of the apoptosis suppressors survivin, Bcl-2 and pro-caspase-3 proteins, and the increased expression of the apoptosis inducer Bax.

CONCLUSION: Our results show that oridonin exhibits an inhibitory effect on the proliferation of RPMI8226 cells and induces apoptosis. This is associated with altering the balance between Bcl-2 and Bax protein expressions and decreased survivin and pro-caspase-3 expressions.

Key words: Oridonin; Multiple myeloma; Apoptosis; Survivin; Bcl-2-associated X protein; Caspase 3

INTRODUCTION

Multiple myeloma (MM) is a malignant tumor in the blood system derived from plasma cells. The effective rate of conventional chemotherapy is approximately 70% for treating MM, with poor clinical complete remission.[1] Most MM gets worse because of easy recurrence and multidrug resistance and becomes an incurable hematological disease. Therefore, the development of potent anticancer drugs is an urgent issue.[1]

Oridonin, a kaurene type tetracyclic diterpenoid compound, is the most important antitumor constituent in Donglingcao (Rabdosia rubescens).[2] Recent research shows that oridonin exhibits good anti-tumor effects in gastric cancer, bladder carcinoma, and melanoma.[3] Its tumor inhibition rate is as high as 41%, and it has been expected to become the "second paclitaxel".[4] A study by the Shanghai Institute of Hematology Research showed that oridonin selectively kills t (8; 21) leukemia cells, suggesting that this compound is a po-

tential candidate for targeted therapy of t (8; 21) acute myeloid leukemia.[5] However, studies on the effect and mechanism of oridonin in MM cells are rare. Therefore, this study was designed to examine the effects of oridonin on the proliferation and apoptosis of the RPMI8226 human myeloma cell line and to investigate the possible mechanism.

MATERIALS AND METHODS

Cell culture and reagents

The RPMI8226 cell line was purchased from the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). The cryopreserved cells were recovered using conventional methods and cultivated in RPMI1640 medium (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin in an incubator at 37 ℃ with 5% CO_2. The medium was changed every 2-3 days. Cells at logarithmic growth phase of second passage were used for further analysis.

Oridonin, purchased from Shaanxi Xuhuang Biotech Company (Xi'an, China) (99.9% purity), was dissolved in dimethyl sulfoxide (DMSO) (Sigma, St. Louis, MO, USA) with a final concentration of 2.5 mmol/L by dilution with PBS (phosphate-buffered saline) (pH 7.4). The solution was stored at − 20 ℃ and protected from light. The required concentration (DMSO final concentration < 0.1%) was obtained by dilution with medium. Thiazolyl blue tetrazolium bromide (MTT) was obtained from Sigma. The Annexin-V/PI double staining assay kit was purchased from Jiankangyuan Pharmaceutical Co., Ltd. (Zhuhai, China). Pro-caspase-3, Bcl-2, Bax, and survivin antibodies were obtained from Zhongshan Biotechnology Company (Beijing, China).

MTT assay

Cells (2×10^5/mL) at logarithmic growth phase were seeded in 96-well plates (HyClone, Logan, UT, USA). Various concentrations of oridonin (5, 10, 15, 20, and 25 μmol/L) were added in 200 μL in each well and cells were cultured for 12, 24, 36 and 48 h in an incubator at 37 ℃ with 5% CO_2. Each of the drug groups was evaluated in quadruplicate. After incubation, 10 μL of 0.5 mg/mL MTT was added to each well and cells were incubated for another 4 h. The supernatant was discarded and 150 μL of DMSO was added with blue-violet crystals completely dissolved by slightly shaking for 10 min. Optical density (OD) values at 490 nm were read by a microplate reader 550 (Biorad, CA, USA). Each experiment was repeated at least three times.

Cell growth inhibition was calculated by the following equation: cell growth inhibition rate (%) = $(OD_{control} - OD_{exp}) / OD_{control} \times 100\%$.

Transmission electron microscope (TEM) analysis

RPMI8226 cells treated with 15 μmol/L oridonin for 24 h were collected and centrifuged. Cells were immediately placed in 2.5% glutaraldehyde at 4℃ for more than 2 h, washed with 0.1 M phosphate buffer for 30 min, fixed in 1% osmium tetroxide for 2 h at 4 ℃, washed for 10 min, dehydrated in gradient ethanol, and embedded in epoxy Epon 812. Semi-thin sections (1-2 μm) were constructed after polymerization and positioned under an optical microscope after methylthioninium chloride staining. Thin sections (50-70 nm) were prepared with an LKB-V type ultramicrotome and stained with acetic acid uranium and lead citrate. Finally, the sections were observed under a Hitachi H-600 TEM operated at 75Kv (Hitachi, Ltd., Tokyo, Japan) and micrographs were obtained.

Assessment of cell apoptosis by flow cytometry

Cells (1×10^6/mL) were seeded in six-well plates (Corning, NY, USA) with 2 mL in each well. We examined three experimental groups: the control group, the 10 μmol/L oridonin group, and the 20 μmol/L oridonin group. All groups were cultured for 12, 24 or 36 h, washed twice with PBS, and then 10 μL of Annexin-V (Invitrogen, Carlsbad, CA, USA) and 5 μL of propidium iodide (PI, Invitrogen) were added. Percentages of apoptotic cells were counted by flow cytometry using FACSCalibur (BD, New York, NY, USA).

Western blot

Cells were treated as indicated and harvested, and total protein was extracted. Protein samples (30 μg) were separated on an SDS-polyacrylamide gel by electrophoresis and then transferred to a nitrocellulose membrane. Nonfat dry milk (5%) was used as a blocking agent for 1 h. The membranes were incubated with primary antibody overnight at 4 ℃, and then with secondary antibody at room temperature for 1 h. Detection of protein bands was performed with a two-color infrared laser imaging system. The bands were scanned and quantitated by densitometry using NIH image software. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control.

Statistical analysis

All data were analyzed by one-way analysis of variance using SPSS 13.0 (version 13.0, SPSS Institute, Chicago, IL, USA). Quantitative data are expressed as mean ± standard deviation. Statistical significance was determined using Student's *t*-test.

RESULTS

Inhibitory effect of oridonin on RPMI8226 cell growth

We first evaluated the effect of oridonin on RPMI8226 cell growth by MTT assay. The results showed a significant reduction of RPMI8226 cell growth after treatment with various concentrations of oridonin in a time- and dose-dependent manner (Figure 1). The inhi-