

The Inhibitory Effect of Oridonin on the Growth of Fifteen Human Cancer Cell Lines

Junhui Chen¹
Shaobin Wang¹
Dongyang Chen¹
Guisheng Chang¹
Qingfeng Xin¹
Shoujun Yuan²
Zhongying Shen³

¹ Department of Oncology, The First Affiliated Hospital of Shantou University Medical College, Shantou 515041, China.

² Pharmacology Laboratory, Institute of Radiation Medicine, Military Medical Sciences Academy of Chinese PLA, Beijing 100270, China

³ Department of Pathology, Shantou University Medical College, Shantou 515031, China.

Correspondence to: Junhui Chen
E-mail: chenjh@stu.edu.cn

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CJCO <http://www.cjco.cn> E-mail: cocr@eyou.com
Tel(Fax): 86-22-2352 2919

OBJECTIVE To study the inhibitory effect of oridonin on the growth of cancer cells.

METHODS Fifteen human cancer cell lines were subjected to various concentrations of oridonin in culture medium. The inhibitory rate of cell growth was measured by the MTT assay, and compared with a negative control and 5-Fu-positive control.

RESULTS The 50% inhibiting concentration (IC_{50}) and maximal inhibition (I_{max}) of oridonin shown by studying the growth of the cancer cell lines were as follows: leukemias (HL60 cells: 3.9 μ g/ml and 73.8%, K562 cells: 4.3 μ g/ml and 76.2%); esophageal cancers (SHEEC cells: 15.4 μ g/ml and 99.2%, Eca109 cells: 15.1 μ g/ml and 84.6%, TE1 cells: 4.0 μ g/ml and 70.2%); gastric cancers (BGC823 cells: 7.6 μ g/ml and 98.7%, SGC7901 cells: 12.3 μ g/ml and 85.7%); colon cancers (HT29 cells: 13.6 μ g/ml and 97.2%, HCT cells: 14.5 μ g/ml and 96.5%); liver cancers (Bel7402 cells: 15.2 μ g/ml and 89.2%, HepG2 cells: 7.1 μ g/ml and 88.3%); pancreatic cancer (PC3 cells: 11.3 μ g/ml and 68.4%); lung cancer (A549 cells: 18.6 μ g/ml and 98.0%); breast cancer (MCF7 cells: 18.4 μ g/ml and 84.7%); uterine cervix cancer (Hela cells: 13.7 μ g/ml and 98.5%).

CONCLUSION Oridonin had a relatively wide anti-tumor spectrum, and a relatively strong inhibitory effect on the growth of the 15 human cancer cells. Inhibitory effects were concentration dependent.

KEYWORDS: oridonin, antitumor effect, in vitro study.

INTRODUCTION

The medicinal herb, Donglingcao, also called Binglingcao, with a scientific name of *Rabdosia rubescens*, is derived from a rabdosia plant of the family Labiatae. As a native of the Yellow River Valley and its southern area, *Rabdosia rubescens* was first harvested in Henan Province of China and used as anti-tumor folk medicine for the treatment of esophageal and cardia cancer. Oridonin, a monomeric ingredient of ent-kaurane diterpene compounds extracted and purified from *Rabdosia rubescens*, has anti-tumor activity and possesses 90% of the active ingredients of *Rabdosia rubescens*^[1]. *Rabdosia rubescens* also contains some other significant chemical compounds including Rubescensin B^[2], Rubescensin C^[3], Rubescensin D^[4] and Rubescensin E^[5]. In the present study, the MTT assay was used to examine the inhibitory effect of various concentrations of oridonin on the growth of 15 common human cancer cell lines. The results given below have been contrasted with a negative control and positive control using 5-Fu.

MATERIALS AND METHODS

Samples and reagents

Oridonin was provided by the Beijing Sanghaobo Science and Technology Corporation as a light yellow powder with a purity of 99%. It was dissolved in dimethyl sulfoxide (DMSO) (purchased from Sigma Co.) to make a stock solution. RPMI 1640 medium, DMEM medium and MTT were all purchased from Sigma Co.(USA).

Cell lines

The following cell lines were maintained and passaged by the Pharmacology Laboratory, Institute of Radiation Medicine, Military Medical Sciences Academy of Chinese PLA and the Research Center of Oncological Pathology, Shantou University Medical College: human leukemias (HL60, K562), esophageal cancers (SHEEC, Eca109, TE1), gastric cancers (BGC823, SGC7901), colon cancers (HT29, HCT), liver cancers (Bel7402, HepG2), pancreatic cancer (PC3), lung cancer (A549), breast cancer (MCF7) and uterine cervix cancer (Hela).

Cell culture

Adherent cells

The cells were inoculated in RPMI 1640 or DMEM medium (supplemented with 100 U/ml of both penicillin and streptomycin) containing 10% fetal bovine serum (FBS) and incubated at 37°C under 5% CO₂ in air. The cell medium was renewed every 2~3 days by using 0.25% trypsin to digest the cells for passaging and collecting.

Cell suspensions

The cells were inoculated in RPMI 1640 medium (supplemented with 100 U/ml of both penicillin and streptomycin) containing 10% fetal bovine serum (FBS), incubated at 37°C with 5% CO₂ in air and passaged every 2~3 days.

MTT assay

Adherent cells

Cells in a logarithmic growth-phase were transferred to 96-well culture plates, each well being loaded with 4~5×10³ cells (100 μl). A cell suspension with 4~5×10⁴ cells/ml was prepared in RPMI 1640 or DMEM cell culture medium containing 10% fetal bovine serum (FBS). The supernatant was discarded after 24 h of incubation. To each well 200 μl of culture medium containing different concentrations of oridonin (0.5,

1, 2, 4, 8, 16, 32, 64, 128 μg/ml) were added, using 4 parallel wells for each concentration. The supernatant was discarded after 72~120 h of incubation. Each well then received 50 μl of fresh MTT serum-free culture working solution (0.5 mg/ml), followed by incubation for 4 h at 37°C. All culture medium supernatant was removed from the wells and replaced with 200 μl of DMSO to solubilize the colored formazan product. The absorbance (OD value) was measured with a microculture plate reader at 570 nm employing a reference 450 nm wavelength after agitating the plates for 15 min.

Cell suspensions

A cell suspension at a concentration of 8~10×10⁴ cells/ml was prepared from logarithmic growth-phase cells in RPMI 1640 medium containing 10% fetal bovine serum (FBS). To each well of a 96-well culture plate, 8~10×10³ cells/well (100 μl) were added followed by incubation at 37°C CO₂ in air for several hours. Four parallel wells were provided for each concentration suspension. After 96 h of incubation, each well received 20 μl of fresh MTT serum-free culture working solution (5 mg/ml) followed by incubation for 4 h at 37°C. A 20% SDS solution (100 μl/well) was added to each well and the plate incubated for 6 h. Absorbance (OD value) was read at 570 nm on a microculture plate reader.

Data processing

Data were expressed as the mean±SD; inhibitory rate=[OD value of the control group—OD value of the observed group]/OD value of the control group ×100%; 50% inhibiting concentration (IC₅₀) was used to express the drug effect, and a measurement of the I_{max} was also determined. The dose-response curve of the growth-inhibiting effect of oridonin on cancer cells was fit to the 4-Parameter Logistic sub-procedure of MicroCal Origin software to determine the IC₅₀ (μg/ml).

RESULTS

The oridonin stock solution was prepared to cover a range of concentrations: 0.5, 1, 2, 4, 8, 16, 32, 64, 128 μg/ml. Culture medium and 5-Fu were used as negative and positive controls. The 15 human cancer cell lines were shown to be relatively sensitive to oridonin. The IC₅₀ values were between 3.9 and 18.6 μg/ml (Table 1), I_{max}: 68.4%~99.2% (Table 2).

Within the set concentration range of oridonin, growth of the 15 human cancer cell lines was inhibited in a favourable dose-dependent manner (Fig.1).

Table 1. Concentration of oridonin inhibiting the growth of 15 human cancer cell lines by 50% (IC₅₀).

Cell lines	IC ₅₀ (µg/ml)
Leukemia	
HL60	3.9
K562	4.3
Esophagus	
SHEEC	15.4
Eca109	15.1
TE1	4.0
Stomach	
BGC823	7.6
SGC7901	12.3
Colon	
HT29	13.6
HCT	14.5
Liver	
Bel7402	15.2
HepG2	7.1
Pancreatic	
PC3	11.3
Lung	
A549	18.6
Breast	
MCF7	18.4
Uterocervical	
Hela	13.7

DISCUSSION

In China, the pharmacologic study of *Rabdosia rubesens* and its active constituents started in the late 70s of the last century. Since then, studies have focused mainly on the anti-tumor effect of oridonin and its mode of action. Based on the studies of the anti-tumor pharmacodynamics of oridonin, in vitro tests have shown that oridonin has an inhibitory effect on such human cancer cell lines as: leukemias (HL60^[6,7], K562^[8,9], HPB-ALL^[10], NB4^[11], Jurkat^[12]); esophageal cancers (CaEs-17^[13]); gastric cancer (MGC80-3^[13]); liver cancer (BEL-7402^[13,14]); nasopharyngeal cancer (CNE^[13,15]); lung cancers (SPCA1^[16], NCI-H520^[11], NCI-H460^[11], NCI-H1299^[11]); breast cancers (MCF7^[11,17,18], MDA-MB231^[11]); ovarian cancers

Table 2. Inhibitory effect of oridonin on the growth of 15 human cancer cell lines cultured in vitro

Concentration (µg/ml)	Inhibition rate (%)														
	HL60	K562	SHEEC	Eca109	TE1	BGC823	SGC7901	HT29	HCT	Bel7402	HepG2	PC3	A549	MCF7	Hela
Negative	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5	9.7	6.7	-	-	5.8	-	-	-	-	-	-	1.1	-	-	-
1	17.9	-9.0	0.9	3.2	-1.6	10.1	6.5	-11.4	5.3	11.1	-4.4	-2.6	0.4	8.3	6.3
2	26.0	-1.7	4.7	1.8	14.6	17.9	4.4	-11.5	4.0	-3.2	-4.4	-14.6	-3.7	5.6	8.4
4	47.1	28.6	4.4	3.9	33.9	32.6	11.7	-8.5	3.1	13.4	6.2	-25.2	-2.5	8.7	9.3
8	58.0	71.0	14.8	12.6	64.1	66.9	18.5	6.7	-1.8	14.8	51.7	3.4	1.4	6.5	10.7
16	73.0	76.2*	57.8	43.7	63.9	98.4	58.9	68.2	62.6	53.6	86.9	61.5	20.3	32.6	76.2
32	73.8*	74.2	98.9	82.2	66.9	98.6	85.7*	97.0	96.5*	86.4	86.9	68.4*	97.1	84.7*	98.4
64	72.9	73.0	99.0	84.5	70.2*	98.6	85.3	97.2*	96.4	89.2*	88.3*	-	97.8	83.2	98.4
128	-	58.9	99.2*	84.6*	-	98.7*	84.6	96.9	96.3	88.9	87.3	-	98.0*	84.1	98.5*
5-Fu (10 µg/ml)	72.0	6.7	65.5	65.1	51.9	89.6	80.0	84.2	95.1	86.3	86.0	-1.4	89.2	58.8	96.7

*I_{max}

(A2780^[18], PTX10^[18]); uterine cervix cancer (Hela^[13,17,19]) and prostatic cancers (LNCaP^[11], DU145^[11], PC3^[11]).

Oridonin is a water-insoluble diterpene compound which is not easily absorbed when taken orally, and there is no preparation for intravenous infusion. Consequently up to the present time, no reports have been published concerning its intravenous usage. With regard to the clinical use

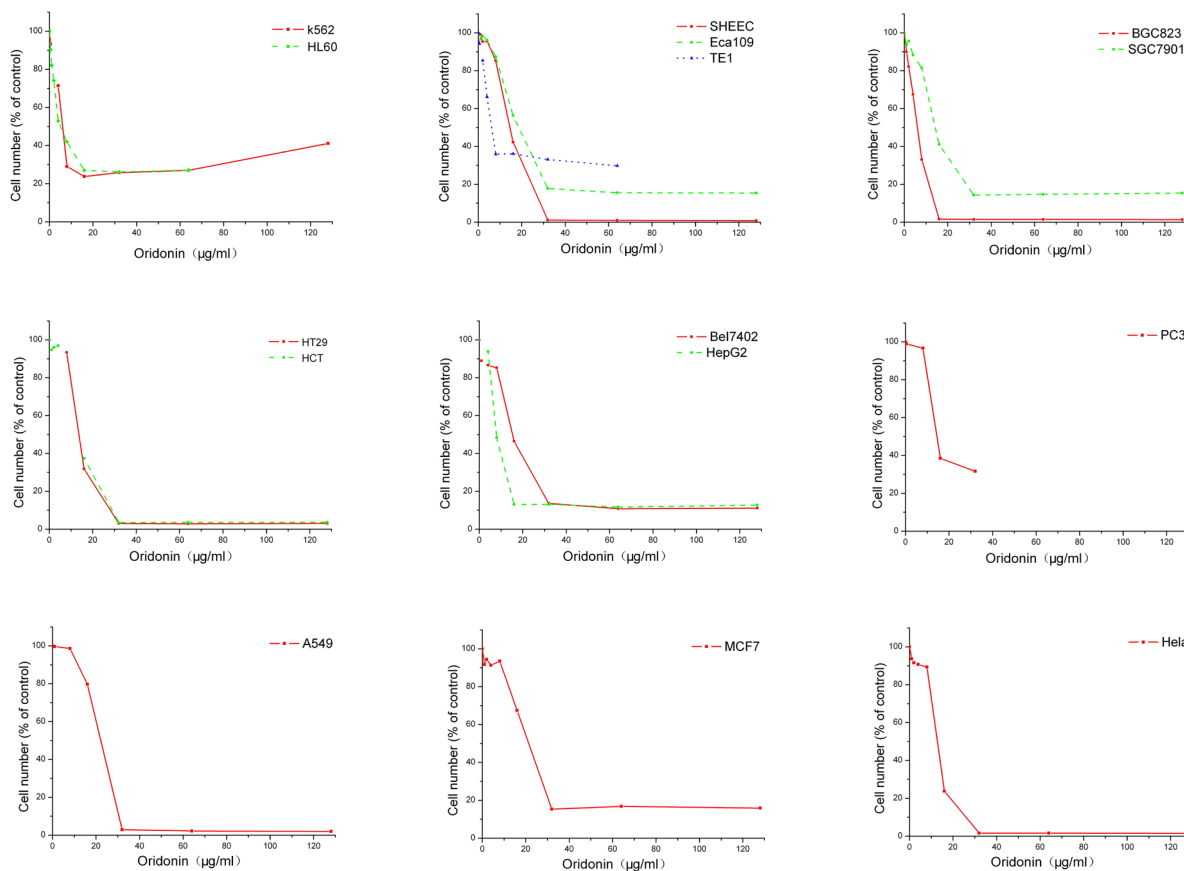


Fig.1. Dose-response curves of the inhibitory effect of oridonin on the growth of 15 human cancer cells cultured in vitro

of oridonin, Wang et al.^[20] employed a crude preparation of *Rabdosia rubesens* (tablet or syrup) to treat 598 patients with malignant tumors, who suffered from cancers at the following locations: 488 esophageal, 44 gastric fundus cardia, 49 primary liver, 35 bladder, 10 breast, 4 colorectum, 3 pancreas, 3 lung and 2 thyroid. Although no statistics were reported for rare tumor types, the clinical effect of *Rabdosia rubesens* was noted for esophageal, gastric fundus cardia, primary liver and bladder cancers. Oridonin is the key component of *Rabdosia rubesens*, so the clinical curative effect of *Rabdosia rubesens* is regarded mainly to reflect the effect of oridonin. Some studies have suggested that the in vitro anti-tumor action of oridonin is like that of a cell cycle-nonspecific drug that inhibits DNA, RNA and protein syntheses^[21-23], but its definite mechanism of action remains unknown.

In order to have a more objective and comprehensive understanding of the type of tumor cells which are sensitive to the in vitro inhibition of growth by oridonin, this study took into account not only the growth of the cells and their relative sensitivity (e.g.

the choice of 15 specific human cancer cell lines), but also covered 9 varieties of common cancers including leukemia and cancers of the esophagus, stomach, colon, liver, pancreas, lung, breast and uterine cervix. The results indicated that oridonin has a relatively wide anti-tumor spectrum, with the 15 cell lines taken from 9 types of human cancers and shown to be relatively sensitive to oridonin, IC_{50} : 3.877 $\mu\text{g/ml}$ ~18.560 $\mu\text{g/ml}$. Among the above 9 common human cancers, all the I_{max} values were greater than 60%. The I_{max} values of esophageal cancer SHEEC, gastric cancer BGC7901, colon cancer HT29, HCT, lung cancer A549 and uterine cervix cancer Hela cell lines reached 95%. The growth inhibitory effect of oridonin on the 15 human cancer cell lines was similar to or greater than that of 5-FU.

A dose-response relationship shown by an in vitro test is valuable in evaluating the anti-tumor effectiveness of oridonin. The concentration of oridonin was studied over a wide range of action. The results showed that when the concentration of oridonin was 0.5 $\mu\text{g/ml}$ ~28 $\mu\text{g/ml}$, proliferation of the 15 cancer

cell lines was effectively inhibited in a dose-dependent manner, and the optimal oridonin concentrations for HL60, SGC7901, HCT, MCF7 were 32 $\mu\text{g/ml}$, for K562, TE1, HT29, Bel7402, HepG2, A549 were 64 $\mu\text{g/ml}$, and 128 $\mu\text{g/ml}$ for SHEEC, Eca109, BGC823 and Hela.

Although, the *in vitro* tests of oridonin can not accurately estimate its clinical effect, in considering the clinical research of Wang et al.^[21], we conclude that oridonin has a favourable potential as an anti-tumor agent. After several years of study, our research group has succeeded in resolving the key technical problems of intravenous infusion. Further study on oridonin pharmacodynamics and its molecular pharmacologic action *in vivo* will be undertaken based on our *in vitro* studies.

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