# THE EFFECT OF IRISQUINONE ON THE GLUTATHIONE SYSTEM AND MRP EXPRESSION OF CISPLATIN-RESISTANT HUMAN LUNG ADENOCARCINOMA CELL LINE ($A_{549}^{DDP}$)

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## ABSTRACT

**Objective: To investigate the Reversal Effect of Irisquinone (ANKA) on Cisplatin-resistant Human Lung Adenocarcinoma Cell Line ($A_{549}^{DDP}$) and the change of MRP expression. Methods: MTT assay, flow cytometry, glutathione reductase recycling assay, RT-PCR were used. Results: None or low cytotoxic concentration of ANKA (10, 20, 30 μmol/L) could increase the sensitivity of $A_{549}^{DDP}$ cells to CDDP by 8.2, 7.9 and 8.9-fold in a dose independent manner. After $A_{549}^{DDP}$ cells was pretreated with 10 μmol/L ANKA for 12 h, CDDP cytotoxicity was increased 9.41-fold. The GSH content of the cells treated by ANKA is reduced significantly ($P<0.001$). The GSTπ protein expression was reduced by ANKA depended on its doses. ANKA also reduced expression of MRP protein, dependent on its dose and treating time ($P<0.001$). MRP mRNA expression was reduced only by 30 μmol/L ANKA ($P<0.05$). Conclusion: The reversal effect of ANKA on $A_{549}^{DDP}$ cell was relative to intracellular glutathione system.**

**Key words: Irisquinone (ANKA), Cisplatin resistance reversal GSH/GST-π**

Cis-diamminedicholoroplatinum (II) (Cisplatin, CDDP) is one of the most successful chemo-therapeutic drugs presently used in the clinic. However, a major limitation to the clinical success of CDDP is the occurrence of resistance to the drug. Cisplatin resistance is a multifactorial phenomenon. This provides an explanation why attempts to reverse or circumvent resistance using CDDP-analogues or combined therapy with modulators of specific resistance mechanisms have had limited success so far. Therefore it is necessary to develop a modulator of resistance that can affect its multiple resistance mechanisms.

Irisquinone (ANKA) is a complex extracted from the seed vessel of Iris Lacet Pall. var., a traditional Chinese Herb[1]. ANKA reduced glutathione (GSH) level and inhibit the repair of DNA singular strand breaks. Therefore, it may reverse cisplatin resistance. We investigated the reversal effect of ANKA on the $A_{549}^{DDP}$ cell line, and its mechanisms.

## MATERIALS AND METHODS

### Materials

Human lung adenocarcinoma cell line $A_{549}$ was donated by cell room of our hospital. Cisplatin resistant human lung adenocarcinoma cell line ($A_{549}^{DDP}$) was produced by our lab. MTT (Thiazolyl blue) was purchased from Sigma Chemical Co.. CDDP was purchased from Kunming sansuguijinsu medicinal Co. Ltd.. Irisquinone (ANKA) was donated by Sansu Inverts Development Co. Ltd.. GSH assay kit was purchased from Nanjing Jiancheng Bioengineering Institute. GST-π monoclonal-antibody and SP assay kit were from Santa Cruz Co. MRP monoclonal-antibody was produced by our lab. MRP primer: 5'-TCTCTCCCGACATGACCGA-

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GG-3' and 5'-CCAGGAATATGCCCCGACTTC-3' and β-actin primer: 5'-ATCATGTTTGAGACCTTCAA-3' and 5'-CATCTCTTGCTCGAAGTCCA-3' were synthesized by Yuanpinghao Biotech Co. Ltd.. DMEM was purchased from Gibco. FCS was purchased from Chuanye Biotech Co. Ltd.

### Cell Culture

Cancer cells were maintained in DMEM medium supplemented with 15% (V/V) heat-inactivated fetal calf serum at 37°C in a humidified atmosphere of 5% $CO_2$ in air. Cells ($1\times10^5$ cells/ml) were passed each 2–3 days. Logarithmic Phase cells were used in experiment.

### MTT Assay

The cytotoxicities of CDDP and ANKA as single agents and in combination were evaluated in $A_{549}$ and $A_{549}^{DDP}$ cells by MTT assay. 100 μl of cell suspension ($0.3–0.5\times10^5$/ml) was plated in 96-well plates in a humidified tissue-culture chamber (37°C, 5% $C0_2$). After 12–18 h, various concen-trations of drugs were added and incubated for 48 h. MTT solution (1 mg/ml) was added and incubated for 4 h. DMSO solution was added and mixed. $OD_{550nm}$ value was measured in SLT. Concentration producing 50% inhibition ($IC_{50}$) was determined by the ability of the cells to convert the tetrazolium MTT salt into a formazan product solubilized in DMSO.

### Determine for Intracellular Level of GSH[3]

$A_{549}^{DDP}$ cells were treated with 0, 10, 20, 30 μmol/L ANKA for 6, 12 h at 37°C in 5% $CO_2$ containing incubator. Cells ($1\times10^6$) of $A_{549}$ and $A_{549}^{DDP}$ treated by drugs were suspended in 100 μl of 10 mmol/L muriatic acid, went through freeze-thaw cycle for 3 times and were centrifuged at 12000 g. 100 μl of supernatant added in 50 μl of 10% sulfhydryl-salicylate solution and were centrifuged at 10000 g. 0.2 ml of supernatant was used to determine the level of GSH with enzyme cycle assay. Deposit was dissolved by 0.15 mol/L NaCl and protein content was determined by Bradford assay.

### Flow Cytometry (FCM)

Samples were treated in the same manner as above. $1\times10^6$ cells were suspended and washed by ice-cold PBS solution. After fixation in ice-cold 70% ethanol (>12 h), cells were incubated by GST-π/MRP monoclonal-antibody at 37°C for 1 h. After washing with ice-cold PBS solution, cells were incubated with FITC-IgG (1:50) at 4°C for 1 h, and then washed by ice-cold PBS solution. Fluorescence Intensity of the expression of GST-π/MRP protein was determined with FCM of American Profil II at 590 nm for excitation, and the results were analyzed by Epso computer.

### Reverse Transcriptase-ploymerase Chain Reaction (RT-PCR)

Samples were treated as same as above. Total RNA was isolated from $2\times10^6$ of cells and reverse transcribed into cDNA, and then amplified by PCR. The products were fractionated by electrophoresis in 1.8% agarose gel. Brightness of the bands was determined by Scanner. Target gene A value/β-actin A value was used as reference of the relative content of target gene mRNA.

### Statistics Analysis

Statistical significance was assessed by *t* test.

## RESULTS

### The Effect of ANKA on CDDP-resistance of $A_{549}^{DDP}$ Cells

The $IC_{50}$ of $A_{549}^{DDP}$ cells treated with CDDP and/or ANKA was studied by MTT assay (Table 1). 10, 20, 30 μmol/L ANKA could reduced the $IC_{50}$ of $A_{549}^{DDP}$ cells from 653.3 μmol/L to 79.75, 82.9, 73.45 μmol/L, approximately 8 times reduction. This showed that ANKA could completely reverse CDDP-resistance of $A_{549}^{DDP}$ cells irrelevant to its dose. Sensitivity to CDDP of $A_{549}^{DDP}$ cells increased the most (reversing 9.41 times) after pretreatment of 10 μmol/L ANKA for 12 h (Figure 1, 2). ANKA had little effect on $IC_{50}$ of $A_{549}$ cells, if any.

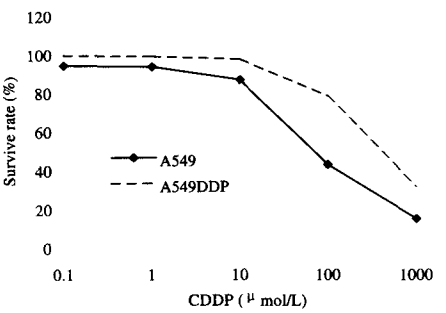



Fig. 1. The cytotoxic effect of CDDP on $A_{549}$ and $A_{549}^{DDP}$cells

### The Effect of ANKA on intracellular content of GSH

As shown in Table 2, the content of GSH in A549DDP cells was as 3 times much as that in parent cells A549 ($P<0.001$). It reduced markedly ($P<0.001$) after treatment with ANKA for 6 or 12 h in a dose-dependent manner (Figure 3).