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*Original Article*

# Induction of apoptosis and reversal of permeability glycoprotein-mediated multidrug resistance of MCF-7/ADM by ginsenoside $Rh_2$

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**Abstract:** Multidrug resistance is a phenomenon that cancer cells develop a cross-resistant phenotype against several unrelated drugs, and permeability glycoprotein derived from the overexpression of *multidrug resistance gene 1* has been taken as the most significant cause of multidrug resistance. In the present study, ginsenoside $Rh_2$ was used to reverse permeability glycoprotein-mediated multidrug resistance of MCF-7/ADM cell line. Effects of ginsenoside $Rh_2$ on the apoptotic process and caspase-3 activity of MCF-7 and MCF-7/ADM cell lines were determined using flow cytometry and microplate reader. Methyl thiazolyl tetrazolium test was conducted to assess the $IC_{50}$ values of ginsenoside $Rh_2$ and adriamycin on MCF-7 and MCF-7/ADM cultures; Rhodamin 123 assay was used to assess the retention of permeability glycoprotein after ginsenoside $Rh_2$ treatment; flow cytometry and real time polymerase chain reaction were used to determine the expression levels of permeability glycoprotein and *multidrug resistance gene 1* in drug-resistant cells and their parental cells after exposure to ginsenoside $Rh_2$. The results showed that ginsenoside $Rh_2$, except for inducing apoptosis, had the ability to reverse multidrug resistance in MCF-7/ADM cell line without changing the expression levels of permeability glycoprotein and *multidrug resistance gene 1*. Our findings provided some valuable information for the application of ginsenoside $Rh_2$ in cancer therapy, especially for multidrug resistance reversal in clinic.

**Keywords:** Ginsenoside $Rh_2$, reversal, glycoprotein, multidrug resistance, MCF-7/ADM

## Introduction

The exposure of cancer cells to a single anticancer drug, including vinka alkaloids, epipodophylotoxins, and anthracyclines, may lead to resistance to a wide range of structural dissimilar drugs [1]. This phenomenon is known as multidrug resistance (MDR) and has been attributed to the over-expression of the *MDR-1* gene which encodes permeability glycoprotein (P-gp). P-gp is a 170-kDa protein, function of which is to export kinds of structural and functional unrelated drugs from cells [2]. Overexpression of P-gp will decrease the accumulation of anticancer drugs within tumor cells [3-7]. Therefore, inhibition of P-gp transporter by pharmacological agents would improve the treatment effect of existing chemotherapy against tumors [8].

As one of the major obstacles for cancer chemotherapy, there are many mechanisms responsible for MDR, including increased drug efflux, DNA repair, and apoptotic signaling pathways [9]. Among all the mechanisms, apoptosis, which plays a key role in controlling cell quantity, is a very important factor of cell death to response to toxicity. Previous study showed that [10] resistance to anticancer drugs was always closely related with a propensity of apoptosis. Generally, apoptosis is characterized with mitochondrial membrane depolarization, cytochrome C release, and caspase activation [11]. In malignancy, the upstream apoptotic signals have been disabled. Although, numerous agents restored the apoptotic process has been described [12], clinical applications are always limited by the deleterious toxicities or the low efficacy. Some more powerful and harmless therapies are imperative.

To solve this problem, researchers and clinician have turned to natural products for help [13-15]. Among kinds of natural products, one of

the most widely used natural tonics in Oriental countries *Panax ginseng*, had gained popularity in West countries during the last decades without any significant toxicity [16, 17]. The major active components of *Panax ginseng* are ginsenosides [18, 19]. Previous studies [20, 21] have shown that crude fractions of ginsenosides extracted from roots of *P. ginseng* could induce a phenotypic reverse transformation in cultured Morris hepatoma cells.

Ginsenoside $Rh_2$ (G-$Rh_2$), found only in red ginseng, is a plant glycoside with a dammarane skeleton and has exhibited potent cytotoxicity against several types of tumors [22]. Hasegawa [23] had hypothesized that G-$Rh_2$ might interact with P-gp because $Rh_2$ had low membrane permeability and potential efflux mediated by ATP-binding cassette transporters, making G-$Rh_2$ mainly distributed in the intestine [24] where P-gp is highly expressed. Moreover, G-$Rh_2$ has been reported to involve in the activation of cyclin A/Cdk2 which induces the apoptosis [25, 26] and reserve the MDR process as well [27]. While more and more attention has been paid to the anti-tumor effect of G-$Rh_2$, the underlying mechanisms of G-$Rh_2$ in regulating tumor cells, especially for the MDR process, still remains unclear.

In the present study, we established adriamycin-resistant human mammary carcinoma cell line MCF-7/ADM in vitro [28, 29] and assessed the effects of G-$Rh_2$ on the apoptotic process and Caspase-3 activity in MCF-7 and MCF-7/ADM cell lines. The cytotoxicity of G-$Rh_2$ on MCF-7 and MCF-7/ADM cultures was estimated by methyl thiazolyl tetrazolium (MTT) test; the retention of P-gp in the two cell lines after treatment with G-$Rh_2$ was illustrated by Rhodamin 123 assay; flow cytometry and real time polymerase chain reaction (RT-PCR) were conducted to determine the expression levels of P-gp and multidrug resistance gene 1 (MDR1) in the two cell lines after exposure to G-$Rh_2$. We hoped that our results would reveal the underlying mechanism of G-$Rh_2$ in regulating the tumor cells, especially for the process of reverse of MDR.

## Materials and methods

### *Preparation of chemicals and MCF-7/ADM cell line*

G-$Rh_2$ (purity > 98%) was obtained from Department of Chemistry, School of Basic Medical Science, Jilin University; adriamycin (ADM), MTT, rhodamin 123, and verapamil were purchased from Sigma.

Human mammary carcinoma cell line MCF-7 provided by Tianjin Medical University Cancer Institute & Hospital was used to induce the resistance against adriamycin. All the procedures were approved by the Ethics Committee of Tianjin Medical University Cancer Institute & Hospital. The cell line was exposed in stepwise increased concentration of ADM to develop the resistant cell line MCF-7/ADM.

### *Inhibition of the growth, induction of apoptosis, and improving of caspase-3 activity in MCF-7 and MCF-7/ADM cell lines by G-$Rh_2$*

MTT assay was performed according to the method of Lau *et al* [30]. Briefly, 50 μL exponentially growing cells ($2 \times 10^5$ cells/ml) were seeded into a 96-well plate in triplicate. Then the cells were treated with increasing concentrations of G-$Rh_2$ [0 μmol/L (control), 0.625 μmol/L, 1.25 μmol/L, 2.5 μmol/L, 5 μmol/L, 10 μmol/L, 20 μmol/L, 40 μmol/L, 80 μmol/L], and each concentration was repeated in triplicate. The experiment was conducted for three times with different G-$Rh_2$ treatment time, including 24 h, 48 h, and 72 h. After G-$Rh_2$ treatment, 5 mg/ml MTT was added to each well and incubated for 4 h at 37°C. The survival rates of different cell lines were measured at 570 nm with a Microplate Reader.

Apoptosis of MCF-7 and MCF7/ADM induced by G-$Rh_2$ was determined using flow cytometry by annexin-V-FITC/PI dual staining. For this experiment, MCF-7 and MCF7/ADM cell lines were prepared as described above, but the working concentrations of G-$Rh_2$ was 0 μmol/L, 10 μmol/L, 20 μmol/L, and 40 μmol/L.

Influence of G-$Rh_2$ on caspase-3 activities in the two cell lines was investigated by measuring the levels of p-nitroaniline (pNA), which was the production of Caspase-3 reacting with its substrate. The cells were treating with 0 μmol/L and 40 μmol/L G-$Rh_2$ for 16 h. The production levels of pNA in different treatments were detected with Microplate Reader at 562 nm. The caspase-3 activity was determined as (production of pNA/incubation time in hours) × (100 μL sample volume/μg protein).

**Table 1.** Inhibiting effect of G-$Rh_2$ on the growth of MCF-7 cell lines (mean ± SD)

| Concentration (μmol/L) | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|
| | OD | Inhibiting Rate (%) | OD | Inhibiting Rate (%) | OD | Inhibiting Rate (%) |
| 0 | 0.399 ± 0.010 | N/A | 0.660 ± 0.041 | N/A | 0.768 ± 0.050 | N/A |
| 5 | 0.360 ± 0.035 | 9.77 | 0.556 ± 0.055 | 15.71* | 0.670 ± 0.007 | 12.72 |
| 10 | 0.363 ± 0.030 | 9.11 | 0.557 ± 0.047 | 15.61* | 0.448 ± 0.034 | 41.60* |
| 20 | 0.363 ± 0.038 | 9.11 | 0.519 ± 0.054 | 21.31* | 0.255 ± 0.018 | 66.83* |
| 40 | 0.206 ± 0.003 | 48.37* | 0.170 ± 0.012 | 74.24* | 0.193 ± 0.016 | 74.82* |
| 80 | 0.160 ± 0.013 | 59.98* | 0.122 ± 0.024 | 81.46* | 0.188 ± 0.008 | 75.51* |

*Significantly different from G-$Rh_2$ concentration of 0 μmol/L.

**Table 2.** Inhibiting effect of G-$Rh_2$ on the growth of MCF-7/ADMcell lines (mean ± SD)

| Concentration (μmol/L) | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|
| | OD | Inhibiting Rate (%) | OD | Inhibiting Rate (%) | OD | Inhibiting Rate (%) |
| 0 | 0.260 ± 0.010 | — | 0.295 ± 0.020 | — | 0.458 ± 0.043 | — |
| 5 | 0.236 ± 0.007 | 9.11 | 0.237 ± 0.016 | 19.86* | 0.379 ± 0.048 | 17.26* |
| 10 | 0.236 ± 0.023 | 9.11 | 0.233 ± 0.007 | 21.22* | 0.345 ± 0.042 | 24.69* |
| 20 | 0.235 ± 0.011 | 9.37 | 0.233 ± 0.026 | 21.11* | 0.289 ± 0.040 | 36.78* |
| 40 | 0.223 ± 0.012 | 14.12 | 0.192 ± 0.017 | 34.99* | 0.246 ± 0.079 | 46.17* |
| 80 | 0.138 ± 0.007 | 46.85* | 0.123 ± 0.016 | 58.24* | 0.202 ± 0.062 | 55.79* |

*Significantly different from G-$Rh_2$ concentration of 0 μmol/L.

**Table 3.** Effect of G-$Rh_2$ on the apoptotic process of MCF-7 and MCF-7/ADM cell lines (mean ± SD)

| Concentration (μmol/L) | Apoptosis (%) | | Necrosis (%) | |
|---|---|---|---|---|
| | MCF-7 | MCF-7/ADM | MCF-7 | MCF-7/ADM |
| 0 | 1.43 ± 0.22 | 1.75 ± 0.23 | 1.59 ± 0.19 | 1.96 ± 0.2 |
| 10 | 1.95 ± 0.47 | 4.36 ± 1.25 | 1.52 ± 0.2 | 1.94 ± 0.28 |
| 20 | 7.31 ± 1.86* | 6.21 ± 0.91* | 3.85 ± 0.49 | 1.83 ± 0.26 |
| 40 | 11.33 ± 0.66* | 26.49 ± 2.42* | 31.9 ± 2.55* | 7.28 ± 0.20* |

*Significantly different from G-$Rh_2$ concentration of 0 μmol/L.

*Effect of $Rh_2$ on the sensitivity of MCF-7/ADM cell line against ADM*

The sensitivity of MCF-7/ADM cell line against ADM after treated with G-$Rh_2$ was determined with MTT assay as described above. The working concentrations of $Rh_2$ used were 5 μmol/L, 10 μmol/L, 20 μmol/L, and 40 μmol/L and MCF-7/ADM cell line was treated for 72 h. Resistance index was calculated as $IC_{50}$ of MCF-7/ADM of each treatment/$IC_{50}$ of MCF-7. The $IC_{50}$ of MCF-7 cell line against ADM was 1.12 ± 0.14 μM, which was measured previously in our lab.

*Detections of expression level of MDR1 and P-gp in MCF-7/ADM influenced by $Rh_2$*

The effect of G-$Rh_2$ on the expression of *MDR1* and P-gp was determined by RT-PCR and flow cytometry. The concentrations of G-$Rh_2$ used here were 0 μmol/L (control), 20 μmol/L, 40 μmol/L, and 80 μmol/L and treatment time was 2 h. And MCF-7 cells were selected as negative control.

Total RNA was extracted by Trizol reagent (Invitrogen life technologies USA) according to operation manual and reverse translated into cDNA template in PE-9600 system. The expression level of *MDR1* was determined with β-actin gene as reference gene (Table S1). Final reverse transcribed reaction mixture of 20 μL includes 10 μL of 2 × Premix Ex Taq™, 0.4 μL of each primer, 0.4 μL of 50 × ROX Reference Dye, 1 μL RNA template, and 7.8 μL $ddH_2O$. The RT-PCR

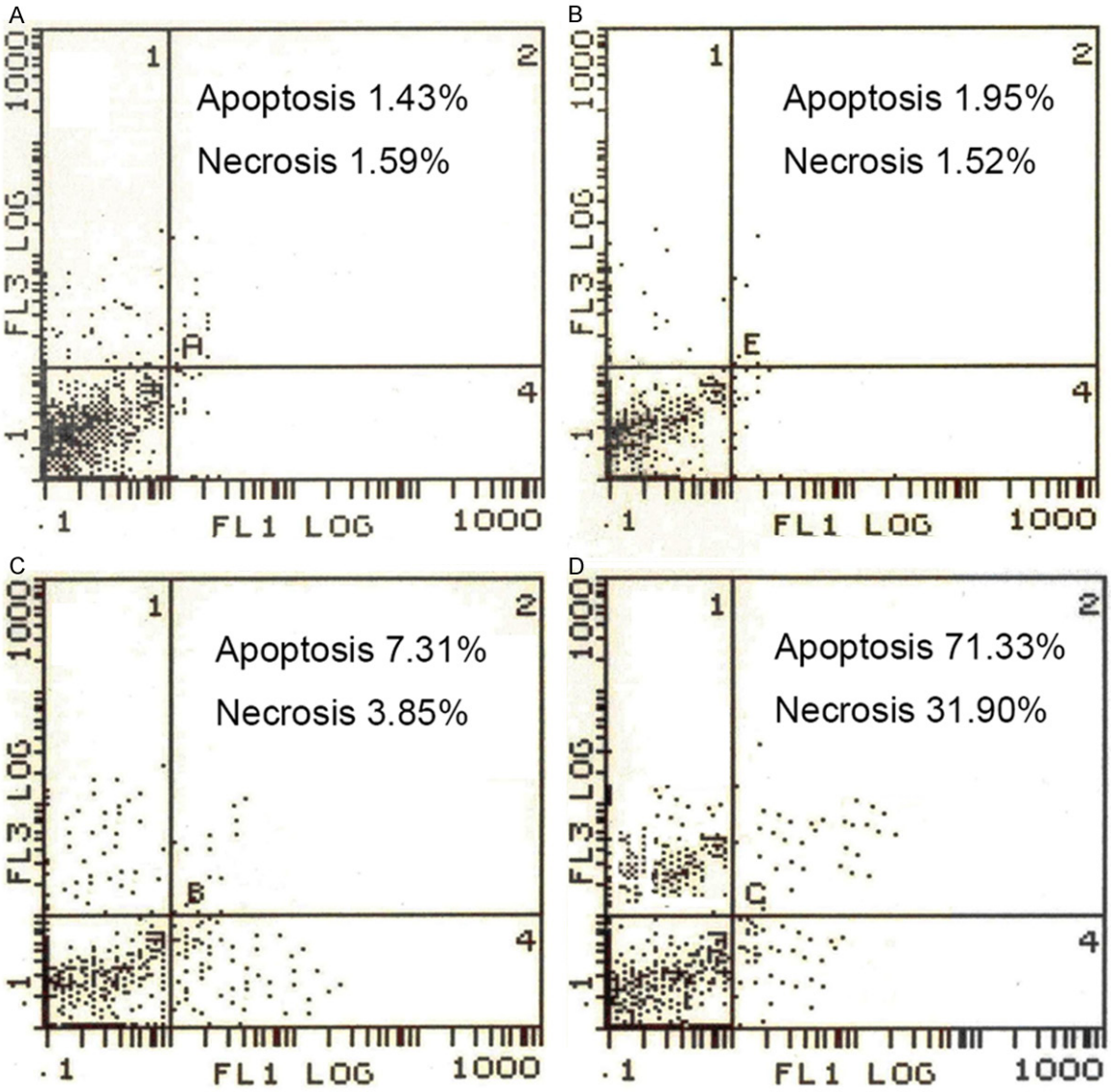


**Figure 1.** Inducing effect of G-$Rh_2$ on apoptotic process of MCF-7 cell lines: A: MCF-7/ADM + 0 μmol/L G-$Rh_2$; B: MCF-7/ADM + 10 μmol/L G-$Rh_2$; C: MCF-7/ADM + 20 μmol/L G-$Rh_2$; D: MCF-7/ADM + 40 μmol/L G-$Rh_2$.

program consisted of 95°C for 15 s, 40 cycles of 95°C for 30 s and 59°C for 30 s, and 72°C for 45 s. The program was conducted in Bio-Rad CFX96TM Real-time system.

The expression levels of P-gp in different cell lines were detected with flow cytometry. Approximately 1 × $10^6$ targeted cells were incubated in RPMI 1640 with 10% FBS at 37°C for 24 h. Then the cells were washed twice with PBS, added with 0.25% pancreatin, and incubated at 37°C for 10 min. After washed by PBS for another two times, the cells were labeled with 20 μlanti-mouse $IgG_1$ and incubated in dark for 30 min before examination with flow cytometer (Beckman coulter. EPICS. XL). Isotype control were set up for MCF-7 and MCF-7/ADM cell lines, respectively. All the experiments were conducted in triplicate.

*Effect of $Rh_2$ on the level of cell retention of rhodamine 123 in MCF-7/ADM cell line*

The retention studies were performed with confluent epithelial monolayers of cell lines grown on 24-well plates. The working concentrations of G-$Rh_2$ used for the treatment of MCF-7/ADM cells were 20 μmol/L, 40 μmol/L, 80 μmol/L, 160 μmol/L, and 320 μmol/L; verapamil was selected as positive control for assessing the

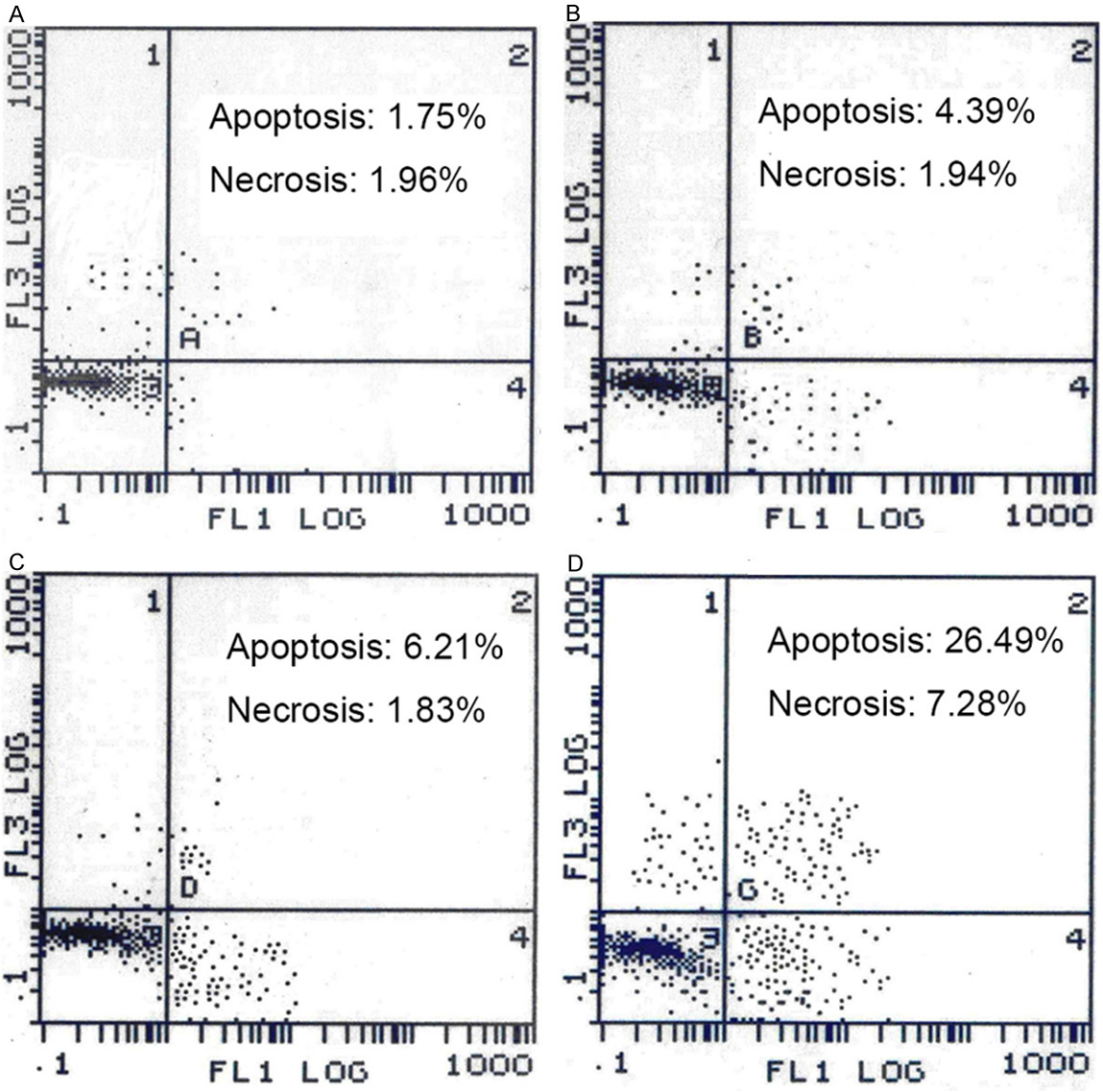


**Figure 2.** Inducing effect of G-$Rh_2$ on apoptotic process of MCF-7/ADM cell lines: A: MCF-7/ADM + 0 μmol/L G-$Rh_2$; B: MCF-7/ADM + 10 μmol/L G-$Rh_2$; C: MCF-7/ADM + 20 μmol/L G-$Rh_2$; D: MCF-7/ADM + 40 μmol/L G-$Rh_2$.

**Table 4.** Effect of G-$Rh_2$ on the production of caspase-3

| | Concentration of Caspase-3 (pmol/μL) | |
|---|---|---|
| | MCF-7 | MCF-7/ADM |
| 0 μmol/L | 13.02 ± 1.79 | 15.50 ± 0.15 |
| 40 μmol/L | 8.07 ± 1.07 | 26.28 ± 0.39* |

*Significantly different from G-$Rh_2$ concentration of 0 μmol/L.

effect of G-$Rh_2$ and the working concentrations of verapamil were 10 μmol/L, 100 μmol/L, 200 μmol/L, and 400 μmol/L; MCF-7/ADM cells without treatment were used as negative control. In brief, cultured cells were washed and then pre-incubated in RPMI 1640 containing 0.25% pancreatin at 37°C for 1-3 min. The RPMI 1640 with 10% FBS was added to inhibit the activity of pancreatin. The cultured cells were incubated with 5 μL Rhodamine 123 at 37°C for 1 h. The concentration of Rhodamine 123 in different cell lines were determined using flow cytometry as described above. All the experiments were conducted in triplicate.

*Statistical analysis*

All the data were expressed in the form of mean ± SD. ANOVA and LSD tests were performed

**Table 5.** Effect of G-$Rh_2$ on the $IC_{50}$ of MCF-7/ADM cell line against ADM

| | Concentration of $Rh_2$ (μmol/L) | | | | |
|---|---|---|---|---|---|
| | 0 | 5 | 10 | 20 | 40 |
| ADM $IC_{50}$ (μM) | 65.43 ± 3.94 | 60.35 ± 1.88 | 58.05 ± 2.95 | 36.02 ± 2.28* | 2.14 ± 2626* |
| Resistance Index | 58.42 | 53.88 | 51.83 | 32.16 | 1.91 |

*Significantly different from G-$Rh_2$ concentration of 0 μmol.

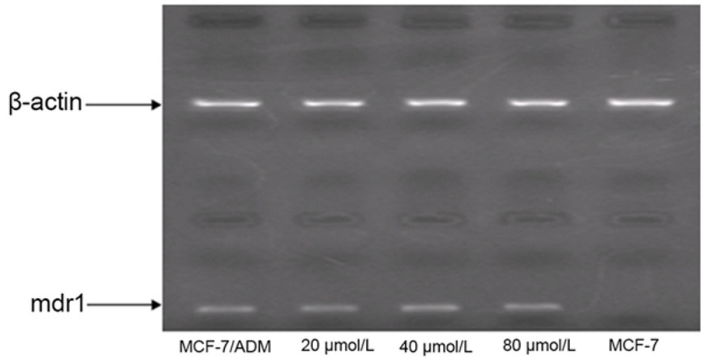


**Figure 3.** Expression of *MDR1* in MCF-7 and MCF-7/ADM cell lines after exposure to different concentration of G-$Rh_2$.

with significant level of 0.05. All the statistical analysis were conducted using SPSS version 16.0 (IBM, Armonk, NY, USA).

## Results

*Effect of G-$Rh_2$ on the growth, apoptotic process, and caspase-3 activities inMCF-7 and MCF-7/ADM cell lines*

The results of MTT assay showed that G-$Rh_2$ had a significant inhibiting effect on the proliferation of MCF-7 and MCF-7/ADM cell lines. The inhibiting effect was time-dependent and significant dose-dependent in the treatment of 48 h and 72 h. For the treatment time of 24 h, we inferred that dose-dependent would be detected with G-$Rh_2$ higher than 80 μmol/L, and more work would be conducted in future studies. Compared with MCF-7/ADM cell lines, the inhibiting effect of G-$Rh_2$ on MCF-7 was more powerful (**Tables 1** and **2**).

The concentrations of G-$Rh_2$ which had influence on the apoptotic process were 20 μmol/L and 40 μmol/L. The effect on inducing apoptosis also differed between MCF-7 and MCF-7/ADM cell lines. For MCF-7 cell line, G-$Rh_2$ resulted in necrosis in most cells while for MCF-7/ADM cell line, G-$Rh_2$ leaded to apoptosis in most cells (**Table 3**; **Figures 1** and **2**).

Significant activation of caspase-3 was detected in MCF/ADM cell line after treatment with 40 μmol/L G-$Rh_2$ but not in MCF-7 cell line (**Table 4**).

*Effect of G-$Rh_2$ on sensitivity of MCF-7/ADM cell line against ADM*

The data showed that the exposure to of G-$Rh_2$ could significantly decrease the ADM $IC_{50}$ at the concentration of 20 μmol/L and 40 μmol/L. The effect existed even at the concentration of 10 μmol/L and 5 μmol/L but statistically insignificant (**Table 5**). The resistance index was reduced to 1.91 under the concentration of 40 μmol/L.

*Effect of $Rh_2$ on expression level of MDR1 gene and P-gp in MCF-7/ADM*

*MDR1* was highly expressed in MCF-7/ADM cells but no expression of *MDR1* was detected in MCF-7 cells (**Figure 3**). After treatment with G-$Rh_2$, the expression of *MDR1* in MCF-7/ADM didn't change significantly (**Figure 3**). The results indicated the existence of MDR process in MCF-7/ADM cell line but not in MCF-7 cell line.

With the flow cytometry detection, it was revealed that the expression level of P-gp was significantly different between MCF-7 and MCF-7/ADM cell lines (**Figure 4**). However, the treatment with G-$Rh_2$ had no influence on the P-gp expression level in the MCF-7/ADM cells (**Figure 5**).

*Effect of G-$Rh_2$ on the level of cell retention of rhodamine 123 in MCF-7/ADM cell line*

The fluorescence intensity of rhodamine 123 within MCF-7/ADM cell line was significantly strengthened after the treatment of G-$Rh_2$ and the effects were dose-dependent at the concentrations < 80 μmol/L and the activity was

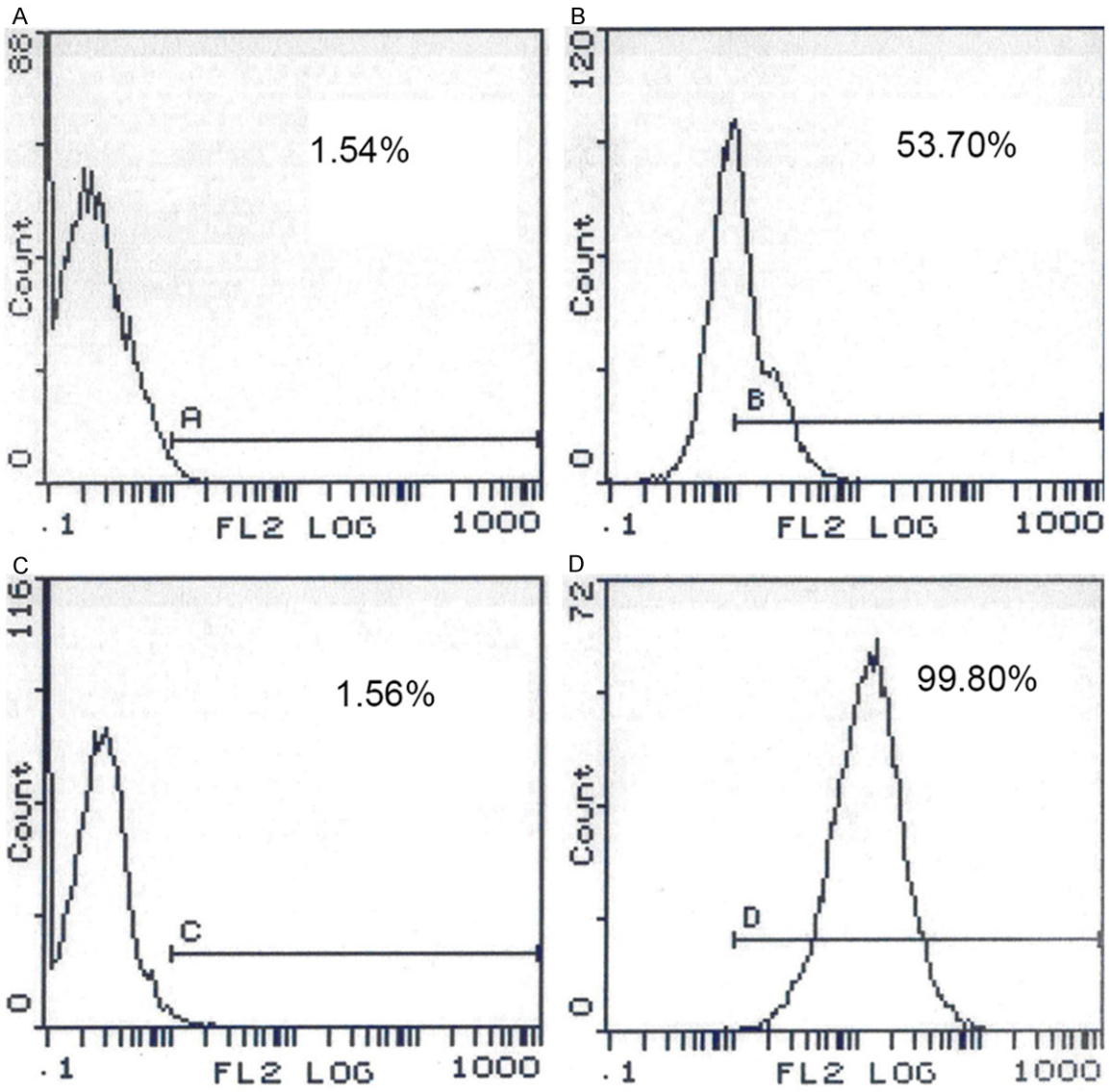


**Figure 4.** Expression of P-gp in MCF-7 and MCF-7/ADM cell lines: A: Isotype control of MCF-7 cell line; B: MCF-7 cell line; C: Isotype control of MCF-7/ADM cell line; D: MCF-7/ADM cell line.

decreased when the concentrations higher than 80 μmol/L (**Figure 6**). In addition, when comparison to verapamil, the improvement of G-$Rh_2$ on cell retention level of rhodamine 123 was more powerful at the concentrations of 40 μmol/L and 80 μmol/L (**Figure 6**).

## Discussion

MDR is one of the major factors resulting in the failure of chemotherapy in treating the tumors. Lots of clinical commonly used medicines, including adriamycin, vincristine, vinblastine, actinomycin, and mitomycin can induce MDR. The underlying mechanism of the occurrence of MDR is complicated [9]. However, increasing studies have confirmed the major characteristic of MDR is the overexpression of *MDR1* which encodes the P-gp. Therefore, inhibition of P-gp transporter by pharmacological agents has been taken as a potential improvement of the existing chemotherapy against tumors.

In the present study, it was found that G-$Rh_2$ was highly effective in inducing the apoptosis and reversing the MDR of adriamycin-resistant human mammary carcinoma cell line MCF-7/ADM *in vitro*. The results of MTT assay showed that the $IC_{50}$ of MCF-7 and MCF-7/ADM after being treated with $Rh_2$ were significantly

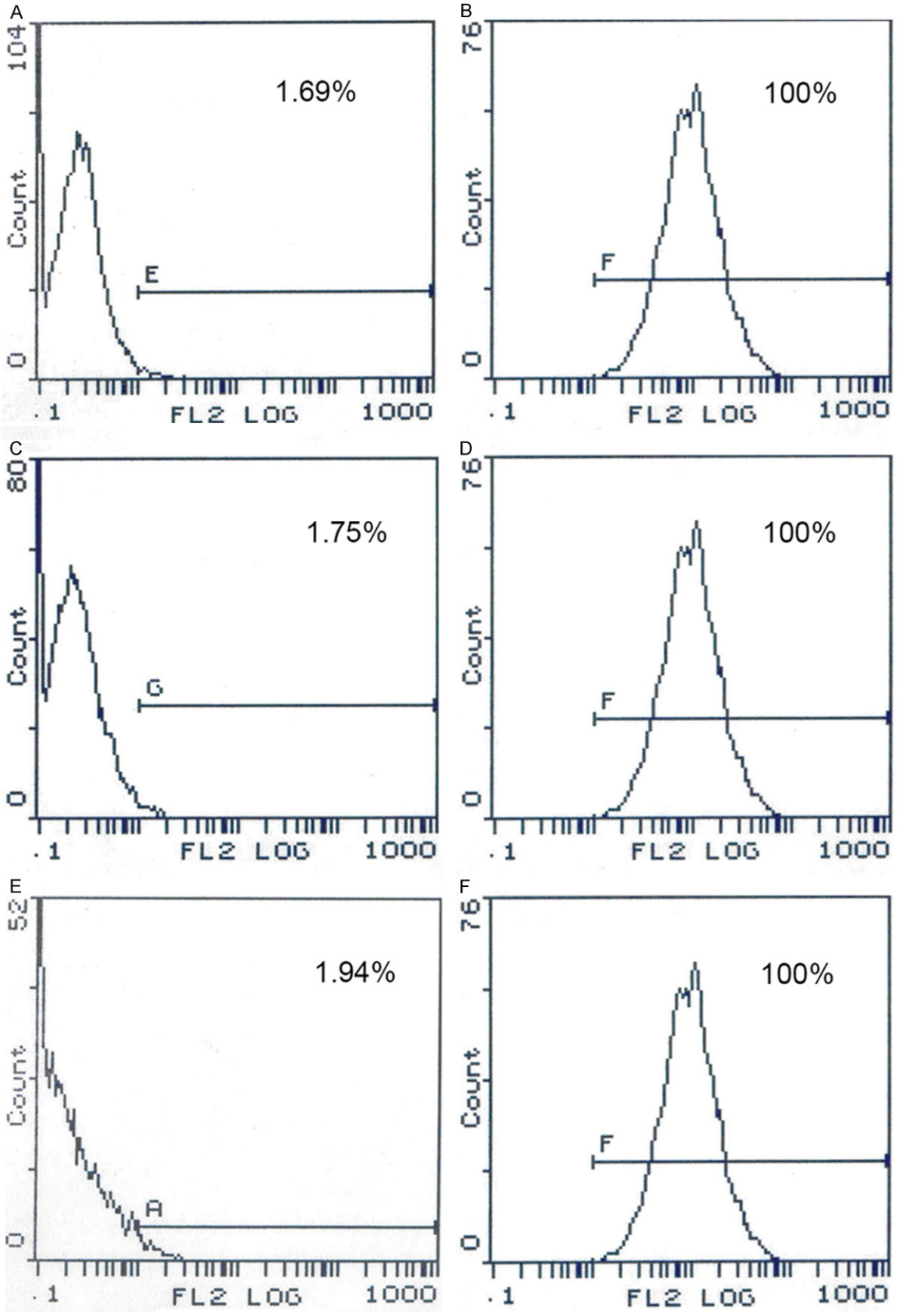
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**Figure 5.** Effect of $Rh_2$ on the expression of P-gp in MCF-7/ADM cell line: A: Isotype control of MCF-7/ADM cell line + 20 μmol/L G-$Rh_2$; B: MCF-7/ADM cell line + 20 μmol/L G-$Rh_2$; C: Isotype control of MCF-7/ADM cell line + 40 μmol/L G-$Rh_2$; D: MCF-7/ADM cell line + 40 μmol/L G-$Rh_2$; E: Isotype control of MCF-7/ADM cell line + 80 μmol/L G-$Rh_2$; F: MCF-7/ADM cell line + 80 μmol/L G-$Rh_2$.

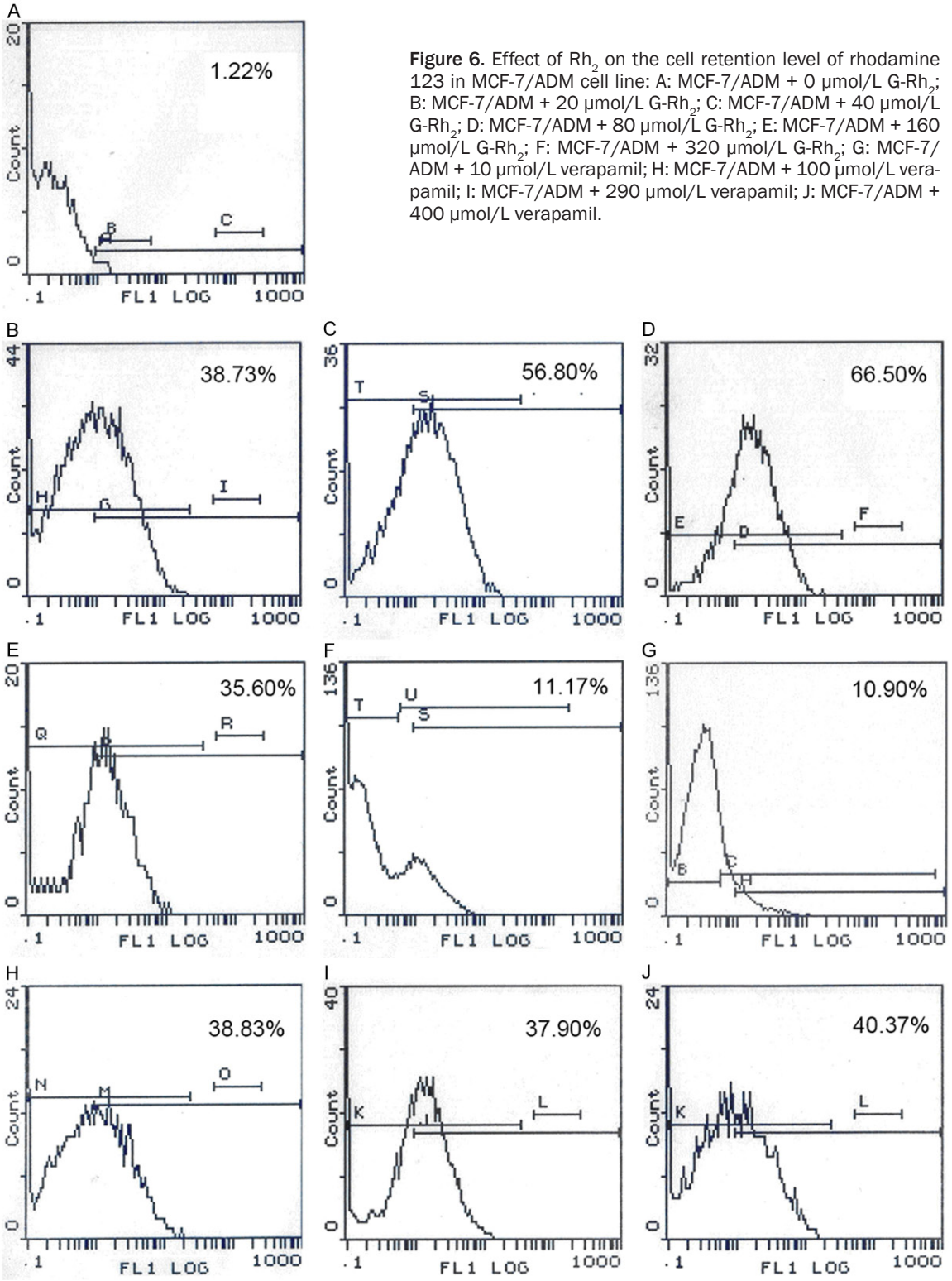


**Figure 6.** Effect of $Rh_2$ on the cell retention level of rhodamine 123 in MCF-7/ADM cell line: A: MCF-7/ADM + 0 μmol/L G-$Rh_2$; B: MCF-7/ADM + 20 μmol/L G-$Rh_2$; C: MCF-7/ADM + 40 μmol/L G-$Rh_2$; D: MCF-7/ADM + 80 μmol/L G-$Rh_2$; E: MCF-7/ADM + 160 μmol/L G-$Rh_2$; F: MCF-7/ADM + 320 μmol/L G-$Rh_2$; G: MCF-7/ADM + 10 μmol/L verapamil; H: MCF-7/ADM + 100 μmol/L verapamil; I: MCF-7/ADM + 290 μmol/L verapamil; J: MCF-7/ADM + 400 μmol/L verapamil.

decreased. The effect also had the characteristics of time and dose-dependence, which showed strong inhibition on the growth of MCF-7 and MCF-7/ADM cells. Moreover, in our experiment, G-$Rh_2$ could active the apoptotic process of different cells with the lowest concentration of 20 μmol/L (**Table 3**; **Figures 1** and **2**). These results seemed to be identical with previous studies conducted in MCF-7 and SK-HEP-1 cells [25, 31, 32], which concluded that G-$Rh_2$ induced the apoptosis of human cells by a mechanism that involves the activation of cyclin A-Cdk2 by caspase-3-mediated cleavage of $p21^{WAF1/CIP1}$. However, the patterns of apoptosis between MCF-7 and MCF-7/ADM cells in the present study were quite different (**Figures 1** and **2**). Moreover, the effect of G-$Rh_2$ on the activity of caspase-3 was also significantly different between the two cell lines, and it was shown that the treatment with G-$Rh_2$ had no influence on the activity of caspase-3 in MCF-7 cell line (**Table 4**). Although we couldn't give a more comprehensive explanation to this difference, it was inferred that for human mammary carcinoma, the apoptotic induction effect of G-$Rh_2$ might not directly act on caspase-3. More detailed work would be conducted in the future to highlight the underlying mechanism.

The ability of G-$Rh_2$ to reverse MDR has been tested with quartz crystal microbalance (QCM) [27]. However, the detail mechanism was not discussed. In the study of $Rg_3$ [33], it was found that the reversal of MDR was attributed to the binding of $Rg_3$ with P-gp. This competitive binding resulted in a decline of the excretion level of other substrates that bind to P-gp. The concentration of $Rg_3$ that significantly inhibited the excretion of Rhodamine 123 (more powerful than the effect of verapamil) in the study of Kim et al was higher than 200 μmol/L. In the present study, our data revealed that $Rh_2$ could increase the sensitivity of MCF-7/ADM against ADM with the lowest concentration of 5 μmol/L (**Table 4**), which was a sign of the reversal of MDR. Compared with $Rg_3$, the concentration G-$Rh_2$ that could significantly inhibit the excretion of Rhodamine 123 was as low as 20 μmol/L, showing a more efficient and safer ability in binding to P-gp (**Figure 6**). However, our results also illustrated a decline trend in the Rhodamine 123 concentration within MCF-7/ADM cells when the concentration of G-$Rh_2$ was higher than 160 μmol/L. This might result from the cytotoxicity of the high concentration G-$Rh_2$ (**Figure 6**). Therefore, we recommended the applicable concentration of G-$Rh_2$ in clinical practice should be lower than 160 μmol/L. In addition, we also confirmed that the treatment with G-$Rh_2$ did not change the expression level of *MDR1* as well as the production of P-gp (**Figures 3** and **5**). The mechanism was similar with that of $Rg_3$. However, except for competitive binding, other studies also infer some other mechanisms for the effect of ginsenosides like $Rh_2$ and $Rg_3$, such as blocking the calcium channel, disabling the chloride channel, and inhibiting the pathway activated by PKC [34-38]. Therefore, further studies need to be carried out in the future to elucidate the underlying mechanism of $Rh_2$ reversing the MDR.

In conclusion, our study demonstrated that as a Chinese medicine monomer, $Rh_2$ had effective ability to induce the apoptosis process in breast tumor cells and reverse the MDR by specifically binding to P-gp. Considering other features including lipid solubility and small molecular weight of G-$Rh_2$, more work should be carried out on G-$Rh_2$ to facilitate the application of this natural product as a promising anti-cancer medicine in the future.

## Disclosure of conflict of interest

None.

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**Table S1.** Detail information of the primers in RT-PCR validation

| Gene ID | | Sequence (5'-3') | Length (bp) | Tm (°C) |
|---|---|---|---|---|
| *MDR1* | Forward | CCATAGCTCGTGCCCTTGTTAGA | 157 | 59°C |
| | Backward | CGGTGAGCAATCACAATGCAG | | |
| *β-actin* | Forward | TGGCACCCAGCACAATGAA | 115 | 59°C |
| | Backward | CTAAGTCATAGTCCGCCTAGAAGCA | | |