Molecular mechanism of matrine from *Sophora alopecuroides* in the reversing effect of multi-anticancer drug resistance in K562/ADR cells

Zhi Chen (ORCID: 0000-0002-7624-3143)[1, 2], Nobuhiro Nishimura[1, 3], Takayuki Okamoto[2], Koichiro Wada[2], Kohji Naora[1]

[1]Department of Pharmacy, Shimane University Hospital, Izumo, Shimane, Japan.

[2]Department of Pharmacology, Faculty of Medicine, Shimane University, Izumo, Shimane, Japan.

[3]School of Pharmaceutical Sciences, International University of Health and Welfare, Okawa, Fukuoka, Japan

Corresponding author:

Kohji Naora

Address: Department of Pharmacy, Shimane University Hospital, Enya-cho 89-1, Izumo, Shimane, 693-8501, Japan.

Email: knaora@med.shimane-u.ac.jp

Fax: 81-853-20-2475

ORCID: 0000-0001-6624-0291

Abstract Multidrug resistance is the main obstacle for current chemotherapies. In this study, we evaluated the reversing effect of matrine, the principal alkaloid derived from *Sophora alopecuroide*, on chemo-resistant leukemia K562/ADR cells. Matrine in a range of the non-toxic concentration was employed in the whole study. IC_{50}s of cancer medicines were tested using WST-8 assay. Drug export and apoptotic rates were examined using flow cytometry. The mRNA and protein expressions were quantified by quantitative real-time PCR and Western blotting, respectively. Our data indicated that matrine had potent reversal properties augmenting cytotoxicity of cancer medicines on K562/ADR cells as well as apoptotic rates induced by doxorubicin. Moreover, matrine inhibited drug exporting activity and expression of ATP-binding cassette subfamily B member 1 (ABCB1) on both mRNA and protein levels. That might result from inhibited NF-kappa B activation, which also led to restored intrinsic apoptosis. These findings suggest that matrine in the non-toxic concentration can suppress ABCB1 drug transport and facilitate the intrinsic apoptosis pathway through the inhibiting effect on NF-kappa B, and has the potential to become an efficient sensitizer for anticancer drug resistance.

Key Words

Apoptosis, ATP-binding cassette subfamily B member 1, Matrine, Multidrug resistance, NF-kappa B.

Abbreviations

Adenosine triphosphate-binding cassette (ABC)

B cell lymphoma 2 (Bcl-2)

Bcl-2 associated X protein (Bax)

Doxorubicin (DOX)

Fetal Bovine Serum (FBS)

Mouse double minute 2 homolog (MDM2)

Paclitaxel (PTX)

Phosphatase and tensin homolog (PTEN)

The concentrations required to inhibit growth by 50% (IC_{50})

1. Introduction

Matrine is the main alkaloid derived from herbal medicine *Sophora alopecuroide* (Fig. 1A). Its anti-tumor effect has been widely concerned in recent years[1]. Many researchers[2,3] have revealed that matrine has a cytotoxic effect on cancer cells due to inhibiting the proliferation of the cells and induce apoptosis. Now an anti-cancer injection containing matrine named “Compound Kushen Injection”[4] has been applied clinically in China. Clinical assessment of efficacy and safety on breast cancer was also implemented[5]. Recently, some researches indicated that matrine might have more valuable properties that sensitize resistant cancer cells to chemotherapeutic agents through suppressing drug exporter ABCB1 and Inhibitor of κB kinase β, which is an NF-kappa B activator[6,7]. However, there is still no systematic research illustrating the mechanism for suppressing the effect of matrine on drug resistance.

Multidrug resistance is a notorious mechanism that induces cancer cells to develop resistance against chemotherapies[8]. Changes in drug export and the apoptosis pathway constitute the primary molecular mechanisms being responsible for the resistance of tumors to anticancer agents[9]. Drug transport associated with multidrug resistance is mainly the export of anticancer drugs from cells inside by adenosine triphosphate-binding cassette (ABC) transporters[10]. Especially, activation of ABCB1 is a common mechanism for cellular resistance to doxorubicin (DOX), paclitaxel (PTX), and vinblastine[11,12]. It is noticeable that a relevant research had found that silencing NF-kappa B can attenuate activation of ABCB1[13]. Deregulated apoptosis may be responsible for multidrug resistance in cancer therapy[14]. Intrinsic apoptosis is involved in the cleavage of caspase-3 and -9 [15]. B cell lymphoma 2 (Bcl-2) proteins family is playing critical roles in regulating intrinsic apoptosis[16]. Therefore, NF-kappa B, as an up-stream protein of Bcl-2 proteins, can inhibit the intrinsic apoptotic pathway[17,18]. Collectively, activation of NF-kappa B can probably simultaneously induce drug export and hamper apoptosis, leading to drug resistance.

In this study, we aimed at clarifying if the non-toxic concentration of matrine could enhance the anticancer drugs in multidrug resistant cells. Moreover, we also attempt to figure out the mechanism by which matrine restores multidrug resistance. Finally, the effects of matrine on the expression and function of drug exporting transporters and apoptosis-relating proteins were examined.

2. Material and Methods

2.1 Cell culture and chemicals

The human leukemia cell line K562 and its multidrug-resistant subtype K562/ADR were purchased from Riken Cell Bank (Saitama, Japan). The cells were maintained in RPMI-1640 medium (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) supplemented with 10% Fetal Bovine Serum (FBS; Biosera, Nuaillé, France) and 1% Penicillin-Streptomycin Solution (FUJIFILM Wako Pure Chemical Corporation) at 37°C under humidified air with 5% CO_2. For sustaining the drug-resistant property, 0.7 μmol/L DOX (Sigma-Aldrich, Delhi, India) was supplemented in the medium for K562/ADR until at least 14 days before each experiment. Matrine was obtained from Indofine Chemical Company, Inc., (Hillsborough, New Jersey, USA).

2.2 Cell viability and reversal effect assay

K562 and K562/ADR cells were placed at an initial density of 5000 cells per well into 96-well plates. After 24 h pre-incubation, cells were implemented with DOX and matrine treatment at indicated concentrations for 48 h. The viable cells were counted using Cell Counting Kit-8 staining according to the manufacturer's instructions (Dojindo Molecular Technologies, Kumamoto, Japan). Absorbance was read by Multi-mode Detector DTX880 (Beckman Coulter, Tokyo, Japan). The concentrations required to inhibit growth by 50% (IC_{50}) were calculated from viability curves using GraphPad Prism 8 (San Diego, California, USA). Reversal fold[19,20] values were calculated using the following formula: Reversal fold = IC_{50} of DOX alone/IC_{50} of DOX in the presence of matrine, to assess the effect of matrine on multi-drug resistance. All experiments were performed three times.

2.3 RNA extraction and real-time quantitative RT-PCR

K562 and K562/ADR cells were seeded into six-well flat-bottom plates and cultured overnight in RPMI-1640 medium supplemented with 10% FBS, followed by being treated with or without DOX or matrine in indicated concentration for 48 h in the dark at 37°C with 5% CO_2. Cells were harvested and washed by ice-cold PBS twice (FUJIFILM Wako Pure Chemical Corporation). Total RNA was extracted with the RNeasy Mini Kit (QIAGEN, Tokyo, Japan). The primers for ABCB1, ABCC1, ABCG2, phosphatase and tensin homolog (PTEN), p53, mouse double minute 2 homolog (MDM2), Akt, and GAPDH were provided by Eurofins Genomics (Tokyo, Japan), and shown in Table S1. The first-strand cDNA was synthesized with the ReverTra Ace qPCR RT Master Mix with gDNA Remover (TOYOBO CO., LTD., Osaka, Japan). Quantitative RT-PCR was performed with KOD SYBR qPCR Mix (TOYOBO CO., LTD.) and measured in the Thermo Cycler DiceTM Real-Time System TP900 (TAKARA Bio Inc., Shiga, Japan). GAPDH was used as a housekeeping control. The relative amount of target mRNA was determined using the $2^{-\triangle\triangle Ct}$ assay[21]. All experiments were triplicated.

2.4 Western blotting

Cell culture was the same as previously described. Whole cell lysates were prepared for western blotting as we previously performed[22]. The samples (2 μg for ABCB1 measurement, 30 μg for others) were equally subjected to 4-15% Mini-PROTEAN TGX Precast Protein Gels (BIO-RAD, Hercules, California, USA) and transferred to Immun-Blot PVDF Membrane (BIO-RAD). After blocking with Blocking One buffer (NACALAI TESQUE, INC., Kyoto, Japan) for 2 h, the membranes were incubated with the indicated antibodies for overnight at 4°C, followed by incubation with horseradish-peroxidase conjugated secondary antibody for 2 h at room temperature. The bands were further dealt with Chemi-Lumi One L solution (NACALAI) and analyzed. The primary antibodies for caspase-8 were obtained from Medical & Biological Laboratories CO., LTD. (Nagoya, Japan) and those for caspase-3, caspase-9, survivin, NF-kappa B p65, p-NF-kappa B p65, Bcl-xL, and Bcl-2 associated X protein (Bax) were from Cell Signaling Technology, Inc. (Danvers, Massachusetts, USA). Primary antibodies for ABCB1 and GAPDH, and secondary antibodies Goat Anti-Rabbit IgG H&L (HRP) and Rabbit Anti-Mouse IgG H&L (HRP) were purchased from Abcam (Cambridge, Massachusetts, USA). The density of bands was measured using a LAS4000 Fluorescence Image analysis system (FUJIFILM, Tokyo, Japan). All experiments were performed three times.

2.5 Apoptosis assay

Cell apoptosis was evaluated with flow cytometry. K562 and K562/ADR cells were seeded into six-well flat-bottom plates and cultured overnight in RPMI-1640 medium supplemented with 10% FBS, followed by being treated with or without DOX or matrine in the indicated concentration for 16 h in the dark at 37°C with 5% CO_2. Cells were harvested and washed twice with PBS, stained with Annexin V-FITC (Abcam) and DRAQ7 (BioLegend, San Diego, California, USA) in the binding buffer (Abcam), and detected by CytoFLEX flow cytometer (Beckman Coulter, Tokyo, Japan) after 5 minutes incubation at room temperature in the dark. Fluorescence was determined with λex 488 nm through FITC channel (525/40 nm) for Annexin V FITC and λex 638nm through APC-700 channel (712/25 nm) for DRAQ7. The early apoptotic cells (Annexin V positive only) and late apoptotic cells (Annexin V and DRAQ7 positive) were quantified. All experiments were performed three times.

Annexin V FITC/ propidium iodide (PI) staining is a typical assay for dividing the live, early apoptotic, and late apoptotic cells using flow cytometry[11,23]. However, in our study, a high concentration of DOX is required to induce apoptosis in our K562/ADR cells due to its strong resistance against DOX caused by constantly co-cultured with low concentration DOX. The concentration of DOX (λex = 480-500 nm, λem = 520-720 nm)[24] used when measuring apoptosis could cause a severe overlapping signal of PI (λex = 493 nm, λem = 636 nm), which seriously influenced the definition of that PI positive or negative (data not shown). DRAQ 7 (λex = 633 nm, λem = 695 nm) is a membrane-impermeable dye that only stains the nuclei of dead or permeabilized cells without the interference of DOX. Therefore we replaced

PI with DRAQ 7 to label the late-apoptotic cells.

2.6 Intracellular rhodamine 123 accumulation

The drug export function of ABCB1 was evaluated by measuring the intracellular accumulation of rhodamine 123. Cell culture was the same as previously described. Ten μmol/L rhodamine 123 (Thermo Fisher Scientific, Tokyo, Japan) was added to the wells and incubated for another 1 h. The cells were harvested and washed twice with ice-cold PBS (FUJIFILM Wako Pure Chemical Corporation). The mean fluorescence intensity (MFI) associated with rhodamine 123 was then determined with λex 488 nm and λem 525nm using CytoFLEX flow cytometer (Beckman Coulter, Tokyo, Japan). All experiments were performed three times.

2.7 Statistics

All the data were statistically analyzed using SPSS 20.0 (Chicago, Illinois, USA), presented as mean and standard deviation, and compared using independent t-test (2-tailed) or one-way analysis of variance. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Determination of the non-cytotoxic concentration of matrine

To test the non-toxic concentration range in K562 cells and its chemoresistant subtype K562/ADR cells, firstly, we examined the cytotoxicity of matrine for 48 h by WST-8 assay. The suppressive effect of matrine on the growth rate of K562/ADR cells was stronger than on the parental cells. IC_{50} of matrine on K562 cells was 3.14 folds higher than on K562/ADR cells. IC_5s of matrine on the two kinds of cells were regarded as the maximum value of the non-toxic concentration (Fig. 1B). Therefore, we set 300 μmol/L as the maximum concentration of matrine, which ensures that over 95% of the two kinds of cells would be viable at the same time, in all the following experiments.

3.2 Reversal effect of matrine on multidrug resistance

Based on the non-toxic concentration range of matrine, cytotoxicity of DOX or PTX in the combination of matrine for 48 h were tested. The reversal fold value represents how much resistance in K562/ADR cells was reversed. As shown in Table 1A, in contrast to K562 cells, K562/ADR cells had exhibited much stronger resistance against DOX. In the combination of 200 and 300 μmol/L matrine, reversal fold values in K562/ADR cells were 2.30 and 2.88, respectively, indicating more than doubled sensitization to DOX. In contrast, no effect of matrine on the IC_{50}s of DOX in the parental cells was observed. This trend was also found in the IC_{50}s of PTX (Table 1B).

3.3 Enhancement of DOX-induced apoptosis by matrine

Effects of matrine in the combination of DOX on the cell apoptosis in the multidrug resistant cells are shown in Fig. 1C and D. Apoptotic rate of cells induced by DOX for 16 h in K562/ADR cells was severely prohibited when compared to K562 cells. Though matrine alone had no significant effect on neither K562 cells or K562/ADR cells, it could dramatically enhance both the early apoptotic rate (Annexin V^+, DRAQ 7^-) and the late apoptotic rate (Annexin V^+, DRAQ 7^+) of cells in the combination of DOX.

3.4 Improvement of intracellular accumulation of rhodamine 123 by matrine

To examine whether matrine could reverse resistance of DOX and PTX in K562/ADR cells through inhibiting the function of ABCB1, we measured the intracellular levels of rhodamine 123, an ABCB1 substrate, in the presence or absence of matrine. As shown in Fig.1 E-H, the accumulation of rhodamine 123 in K562/ADR cells was much lower than that in K562 cells. Furthermore, matrine improved the intracellular levels of rhodamine 123 in both cell lines. However, improvement in K562/ADR cells (increased by 133.6%) was much more significant than in K562 cells (increased by 9.6%).

3.5 Suppressed expression of ABCB1, ABCC1 and ABCG2 in matrine-treated cells

Messenger RNA levels of ABCB1, ABCC1 and ABCG2 are shown in Fig. 2A. Expression of ABCB1 mRNA in K562/ADR cells was 6.3 folds higher than that in K562 cells, while increased expression was not found in ABCC1 and ABCG2. The ABCB1 mRNA level in

K562/ADR cells was decreased to 87.5% by exposure to 300 μmol/L matrine.

Effects of matrine on the protein level of ABCB1 are shown in Fig. 2B and C. Protein expression of ABCB1 in K562/ADR cells was 4.85 folds high as that in K562 cells, which was decreased by 25.2% under the treatment of 300 μmol/L matrine.

3.6 Inhibited activation of NF-kappa B by matrine

The protein level of NF-kappa B in the K562/ADR cells are shown in Fig. 2D-F. Under the treatment of DOX for 48 h, NF-kappa B expression was 25% higher and phosphorylated NF-kappa B was 30% (though not statistically significant) higher in K562/ADR cells in comparison to K562 cells. In the combination of 300 μmol/L matrine, phosphorylated NF-kappa B was decreased to 75% in the resistant cells, which recovered to the level in the parental cells.

3.7 Effects of matrine on the apoptotic proteins

As an executor of apoptosis, protein expression of cleaved caspase-3 in K562/ADR cells was approximately one-third of that in K562 cells. With the treatment of 300 μmol/L matrine, the expression in the resistant cells was improved by 32% (Fig. 3A and B). As for an intrinsic apoptotic factor caspase-9, in K562 cells, the protein expression level of caspase-9 was 15% lower and cleaved caspase-9 was 85% higher than that in K562/ADR cells. In the combination of 300 μmol/L matrine, the expression of caspase-9 and cleaved caspase-9 in resistant cells were improved by 19% and 42%, respectively (Fig. 3C and D). Survivin, which can inhibit the caspase-3 and caspase-9 activation, was significantly suppressed by matrine in resistant cells, although the parental cells had the higher expression level than the resistant cells (Fig. 3E and F). Expression of Bcl-xL, a suppressor of caspase-9 activation, was lower in K562 cells than in K562/ADR cells. Matrine down-regulated the expression of Bcl-xL into 69% in the resistant cells, which was comparable to that of the parental cells (Fig. 3G and H).

4. Discussion

Multidrug resistance is the main obstacle in chemotherapies nowadays. Many relevant studies have endeavored to search for molecular targets to solve this conundrum[8,25]. Several chemo-medicines like verapamil was found positive side-effects that suppressing drug export and recover apoptosis[26]. However, it is not used practically at the clinical level. It is possible that natural products may effectively suppress multidrug resistance in various pathways. One of the advantages of the natural product is comparatively safe to the human body, those that are effectively suppressing multidrug resistance have the potential to be further studied and evaluated[11,19,27]. No doubt, the safety of medicine is an essential premise for its effectiveness. Rather than several studies using IC_{20} as the maximum concentration of multidrug resistance reversing agents[19,28], we employed a maximum concentration even lower than IC_5. In this study, we first assessed the properties of matrine that preventing drug efflux and facilitating apoptosis, and elucidated their relationships.

Firstly, our purpose in this study is to elucidate the mechanism of ubiquitous anti-drug resistance in cancer cells, and how matrine suppressed it. K562 and K562/ADR cells were chosen as the model because they have been frequently used in the many researches to elucidate possible mechanisms for multidrug resistance, especially ABC transporters[19,20]. As anticancer drugs, we utilized DOX and PTX, which are well known to be ABCB1 substrates and induce multidrug resistance[19]. In the experiment on the reversal effect of matrine, the non-toxic concentration of matrine increased the sensitivity to DOX and PTX in resistant K562/ADR cells with the reversal fold values of 2.88 and 3.12, respectively (Table 1A and B). These reversal effects were comparable to the other natural products reported in the previous researches[19,20].

ABCB1 has attracted much concern for its drug efflux properties[9,29]. Rhodamine 123 is a well known specific ABCB1 substrate. It has been utilized as an indicator of ABCB1 function in many relevant researches[11,12,30]. Matrine at the non-toxic concentration improved intracellular rhodamine 123 levels, especially in K562/ADR cells (Fig. 1E-H). This can be well explained by the decreased expression of ABCB1 mRNA and protein by matrine (Fig. 2A). However, we were facing two questions: first, is ABCB1 the only responsible drug exporting factor? Second, is there any up-stream factors that are regulating ABCB1 expression?

Except for ABCB1, ABCC1 and ABCG2 have been reported as the responsible membrane exporters for anticancer drug resistance. In Fig. 2A, unlike ABCB1, no significant elevation of ABCC1 and ABCG2 was found in our resistant cells with or without matrine treatment. These results suggest that suppression of ABCB1 expression by matrine is one of the mechanisms for the increased sensitivity to anticancer drugs. Though the ABCB1 level in K562/ADR cells was significantly higher than that in K562 cells, indicating high ABCB1 expression is a crucial factor for the drug resistance in this study, we can thus prove the correlation between ABCB1 and resistance of K562/ADR cells with ABCB1 knockdown cells in future works.

For tracing up-stream modulators[31,32] of ABCB1, mRNA levels of PTEN, p53, Akt, and MDM2 were also examined, but we could not find any evidence that they were responsible for ABCB1 down-regulation in K562/ADR cells treated with matrine (Fig. S1). On the other hand, we found over-expressed NF-kappa B and its phosphorylated form, which believed to be promotors of ABCB1[13,33], in K562/ADR cells (Fig. 2D-F). Chen et al. reported that PDTC, a specific inhibitor of NF-kappa B, could down-regulate expression of ABCB1 in Caco-2 vbl cells, revealed the up-stream modulating status of NF-kappa B on ABCB1[33]. Our results showed that matrine suppressed phosphorylation of NF-kappa B in K562/ADR cells, implying that it could be responsible for the suppressed ABCB1 level. However, we did not show enough evidence to explain whether matrine down-regulated ABCB1 through inhibiting NF-kappa B, or inhibited ABCB1 directly in this research. Knockdown factors or inhibitors of NF-kappa B can provide more direct and convincible evidence to reveal its relation with ABCB1. Because NF-kappa B is also known to be an up-stream inhibitory factor for the intrinsic apoptosis pathway[17,18], it was necessary to test the influence of matrine on apoptosis-relating proteins.

Matrine in the non-toxic concentration enhanced apoptosis induced by DOX in K562 and K562/ADR cells though it was not effective alone (Fig. 1C and D). To elucidate the mechanism, we examined several related proteins in the intrinsic and extrinsic apoptotic pathways. In the intrinsic apoptotic pathway, inhibited activation of caspase-9, as well as downstream executor caspases-3, can also usually lead to cancer treatment failures. Caspase-9 can be activated by pro-apoptosis protein Bax, and anti-apoptosis protein Bcl-xL. Survivin can prohibit the process of caspase-3 activation[15,34]. According to our findings (Fig. 3), activations of caspase-3, caspase-9 were inhibited and Bcl-xL were activated in our resistant cells. Matrine inhibited Bcl-xL and survivin expression and lead to the reactivation of caspase-3 and caspase-9, partly recovered the intrinsic apoptosis. Although Bax expression did not change by matrine (Fig. S2), this is consistent with up-regulated caspase-9. In total, these changes in expression levels of apoptosis-relating proteins in the intrinsic pathway are considered to be another mechanism for the increased sensitivity to anticancer drugs by matrine.

The extrinsic apoptotic pathway is triggered by the interaction of exposed death receptors located on the cell surface. The activation of initiator caspase-8 can further activate the down-stream executor like caspase-3, and sabotage critical substrates for cell viability, urging cell death[15]. Nevertheless, we could not find the difference of the caspase-8 level, which is vital in the extrinsic apoptotic pathway (Fig. S3). These results indicate that matrine does not affect the extrinsic apoptosis pathway but affects the intrinsic pathway.

NF-kappa B is an up-stream factor that activates Bcl-xL and thus inhibits caspase-9, which plays a crucial role in handicapping the intrinsic apoptotic pathway[17,18]. Because matrine suppressed the activation of NF-kappa B (Fig.2 D-F), we assumed that suppressed anti-apoptotic proteins and recovered activities of pro-apoptosis protein were involved with NF-kappa B inactivation and thus recover the intrinsic apoptotic pathway exclusively. Our hypothesis is supported by several relevant research. Shao et al. implied suppression of inhibitor of kappa B kinase β by matrine might lead to suppressed NF-kappa B activation[35]. Zhou et al. found matrine induced cell death in HepG2 cells through facilitating the intrinsic apoptotic pathway[3]. Luo et al. reported matrine suppressed survivin expression in NCI-H520/TAX25 cells, also implying it could facilitate the intrinsic apoptotic pathway[6]. Zhou BG et al. indicated matrine could facilitate intrinsic apoptotic pathway by down-regulating anti-apoptotic factor Bcl-2[7].

Although the effect of matrine on either ABCB1 or apoptosis-related proteins are small (about 20-30% compared to control), the collective effect of them can lead to a noticeable weakened multi-drug resistance, which can be observed through IC_{50}s.

Natural products derived from herbal medicines are broadly synthesized into compounds with anti-cancer or multidrug resistance reversing activities[36]. It is important that detailed mechanisms for anti-cancer and multidrug resistance reversing properties as well as safety in these compounds are investigated.

5. Conclusion

In conclusion, our study demonstrated that matrine in the non-toxic concentration resensitized multidrug resistant K562/ADR cells through two ways: re-activating apoptosis and inhibiting drug efflux. Matrine can down-regulate phosphorylation of NF-kappa B to recover pro-apoptotic factor and suppress anti-apoptotic factors, leading to facilitated intrinsic apoptosis. In addition, matrine can down-regulate ABCB1 expression to induce diminished drug efflux, which may be also related to the suppressed NF-kappa B.

Figure/Table Legends

Fig. 1 The effect of matrine in non-toxic concentration on apoptosis and rhodamine 123 accumulation in K562 and K562/ADR cells. A: Chemical structure of matrine. B: Evaluation of non-toxic concentration of matrine carried out by WST-8 assay. C and D: Enhancement of DOX-induced apoptosis by matrine, measured by Annexin V FITC and DRAQ 7 staining with flow cytometry. E, F, G, and H: Change in intracellular rhodamine 123 (Rho 123) accumulation by matrine, examined by flow cytometry. All the data were expressed as mean and SD of three independent experiments (B, C, F, and H; *P < 0.05, **P < 0.01, ***P < 0.001; ANOVA or t-test).

Fig. 2 Effects of matrine on drug exporters and activation of NF-kappa B. A: ABCB1, ABCC1 and ABCG2 mRNA expressions measured by quantitative real-time PCR analysis, $2^{-\triangle\triangle Ct}$ assay. B and C: ABCB1 protein expressions examined by western blotting. D, E, and F: NF-kappa B and p-NF-kappa B protein expressions examined by western blotting. All the data were expressed as mean and SD of three independent experiments (A, C, and F; *P < 0.05, **P < 0.01; ANOVA).

Fig. 3 Effects of matrine on the intrinsic apoptosis relating proteins. A and B: caspase-3, C and D: caspase-9, E and F: survivin, G and H: Bcl-xL. Protein expressions were examined by western blotting. All the data were expressed as mean and SD of three independent experiments (B, D, F, and H; *P < 0.05, **P < 0.01; ANOVA).

Table 1 Determination of IC_{50}s of DOX and PTX in the combination of different concentrations of matrine. Cell viabilities were examined by WST-8 assay. IC_{50}s and IC_5s were calculated. Reversal fold = IC_{50}s for cells treated with matrine/ IC_{50}s for cells treated without matrine. Each value of IC_{50} represented as mean and SD of three independent experiments.

Table 1 Determination of IC_{50}s of DOX and PTX in the combination of different concentrations of matrine

A. DOX

Cells	Concentration of matrine (μmol/L)	IC_{50} of DOX (μmol/L)	Reversal fold
K562	0	2.75 ± 0.9	
	200	2.38 ± 1.11	1.16
	300	2.77 ± 1.66	0.99
K562/ADR	0	73.63 ± 2.37	
	200	31.99 ± 4.31	2.3
	300	25.53 ± 1.28	2.88

B. PTX

Cells	Concentration of matrine (μmol/L)	IC_{50} of PTX (nmol/L)	Reversal fold
K562	0	793 ± 149	
	200	692 ± 77	1.15
	300	629 ± 37	1.26
K562/ADR	0	7990 ± 561	

200	2789 ± 284	2.86
300	2563 ± 196	3.12

Figures

Fig. 1

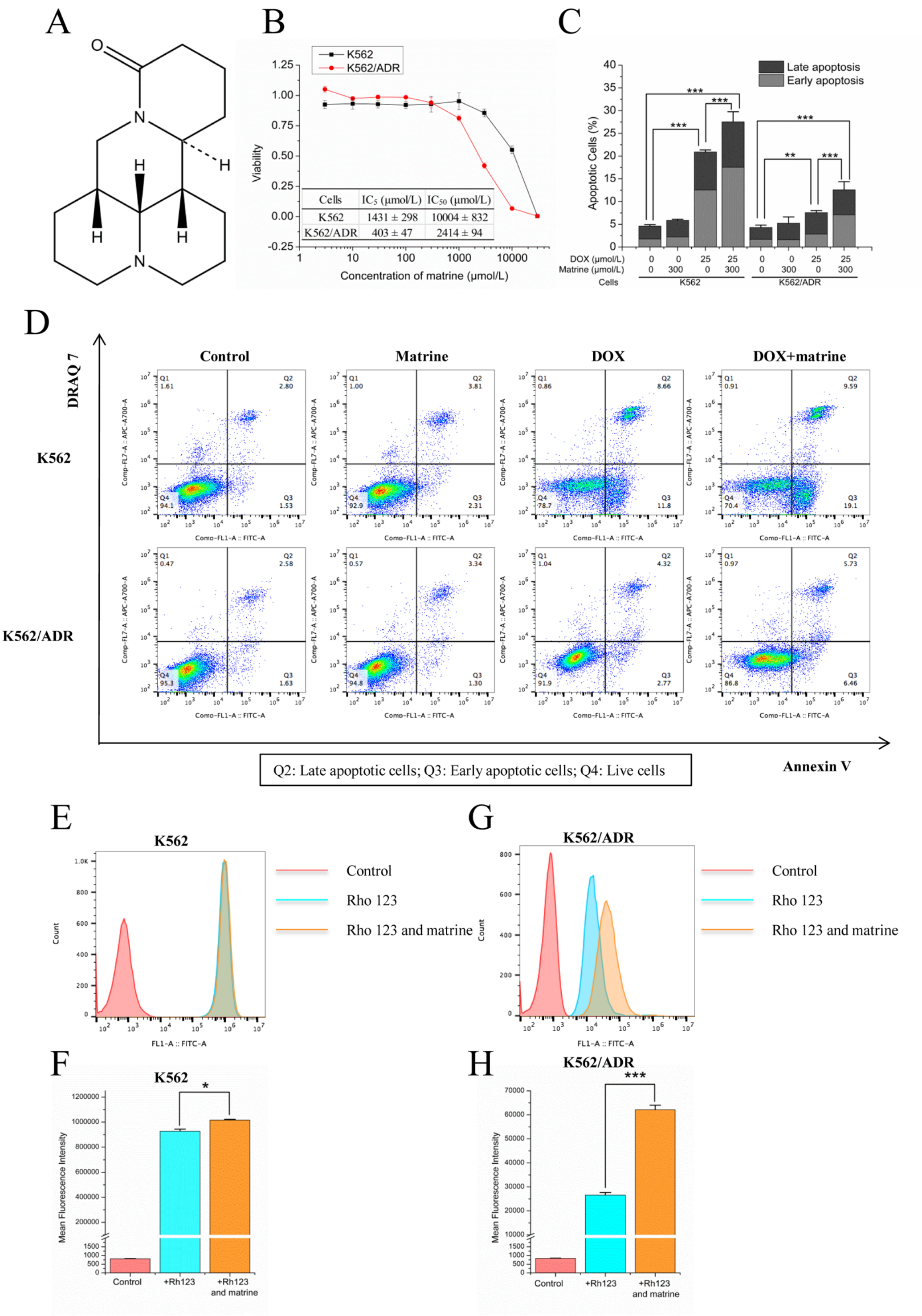


Fig. 2

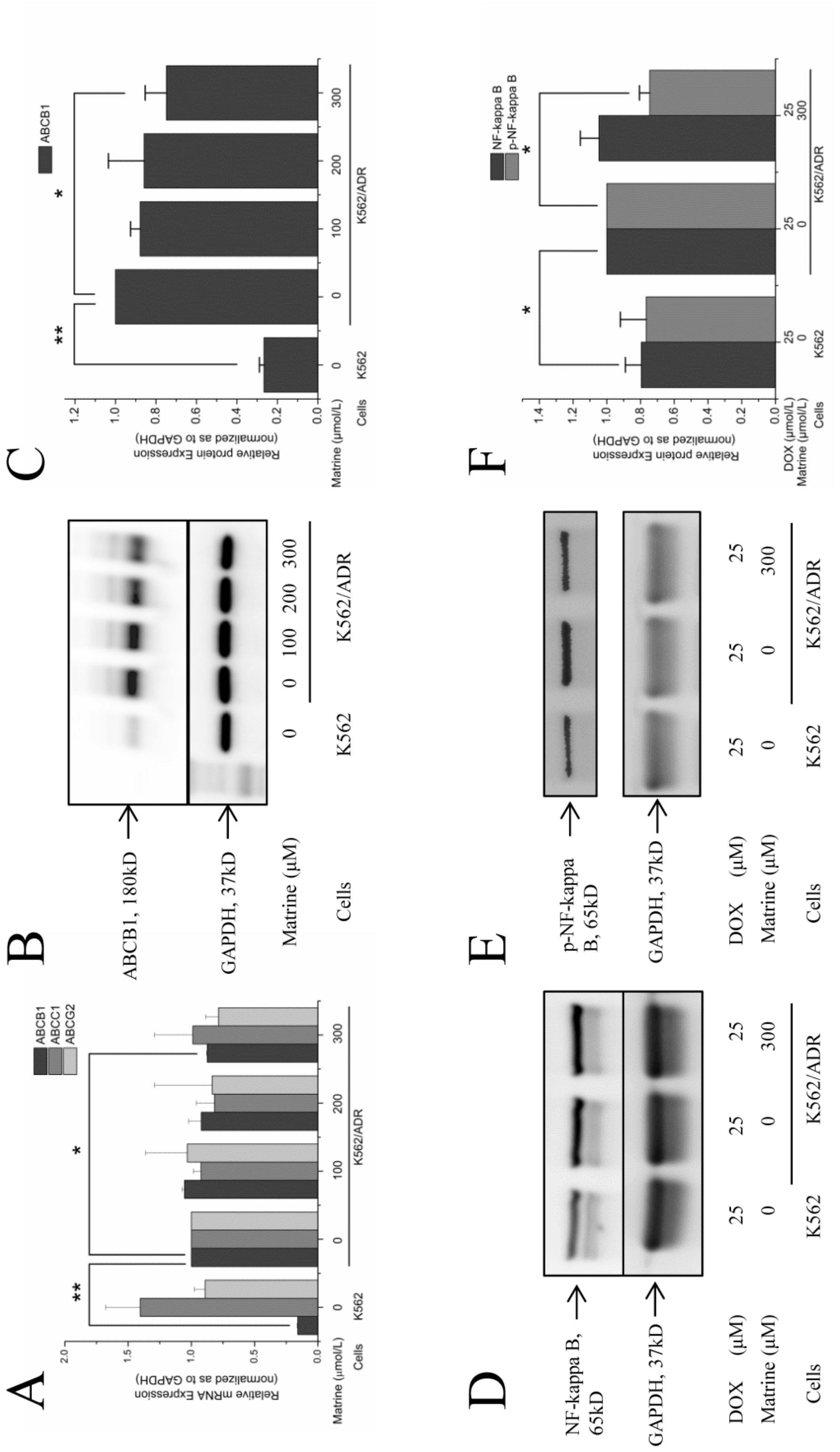
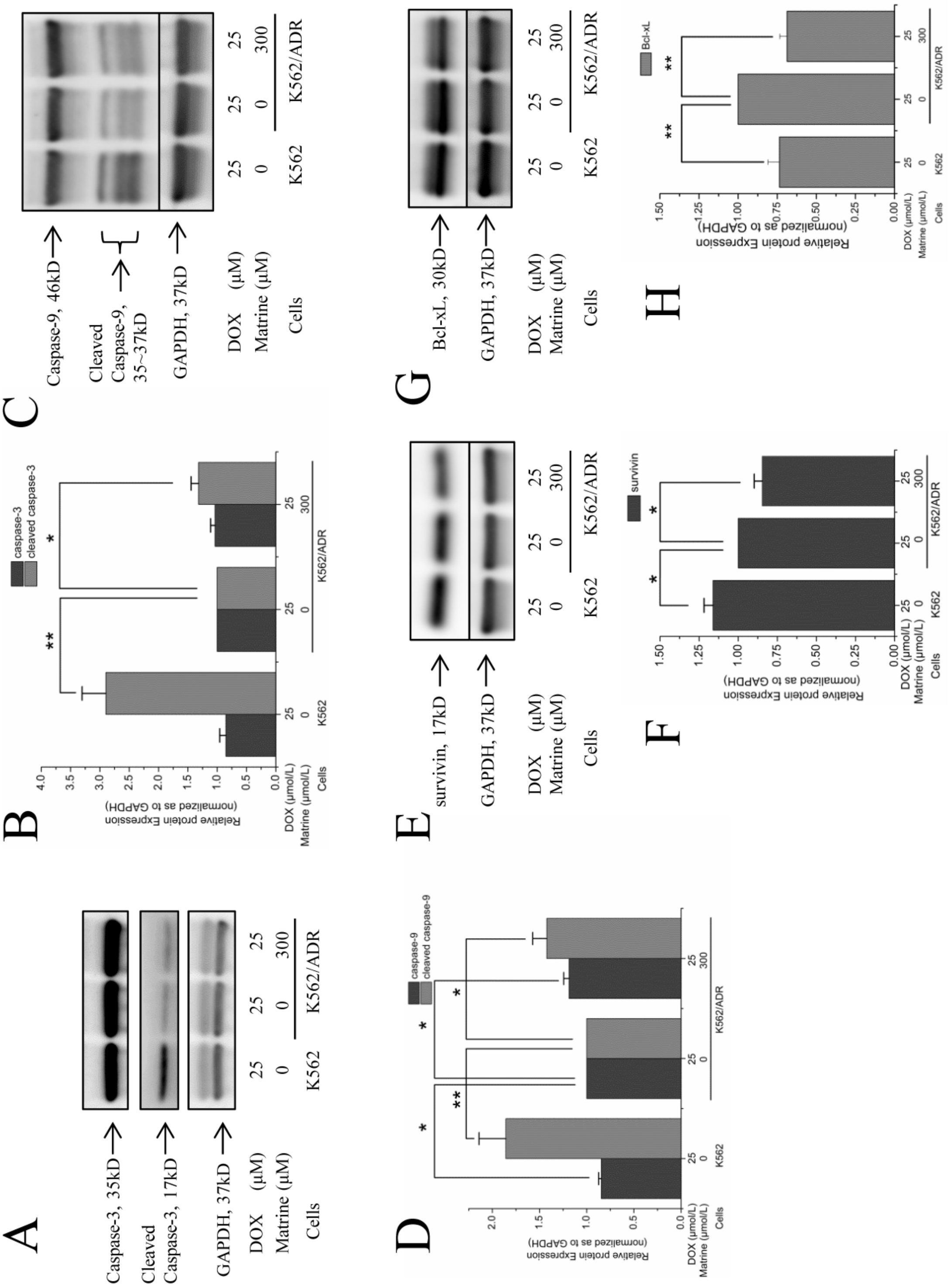

A
B
C
D
E
F
Relative mRNA Expression (normalized as to GAPDH)
Relative protein Expression (normalized as to GAPDH)
ABCB1
ABCC1
ABCG2
Matrine (μmol/L)
DOX (μmol/L)
Cells
K562
K562/ADR
ABCB1, 180kD
GAPDH, 37kD
Matrine (μM)
NF-kappa B, 65kD
p-NF-kappa B, 65kD
DOX (μM)
NF-kappa B
p-NF-kappa B

Fig. 3


A
Caspase-3, 35kD
Cleaved Caspase-3, 17kD
GAPDH, 37kD
DOX (μM)
Matrine (μM)
Cells
K562
K562/ADR
B
caspase-3
cleaved caspase-3
Relative protein Expression (normalized as to GAPDH)
C
Caspase-9, 46kD
Cleaved Caspase-9, 35~37kD
D
caspase-9
cleaved caspase-9
E
survivin, 17kD
F
survivin
G
Bcl-xL, 30kD
H
Bcl-xL

Data availability

The data used to support the findings of this study are mostly included within the article or supplementary files. Further details are confidential according to the rules about the restrictions on data sharing in our institute, which is financially supporting this study (see below). For reviewing progress or potential readers' interest, the first author (Zhi Chen) would provide further details on request, after the approval of the institute.

Conflict of interest statement

The authors have no conflict of interest.

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