ORIGINAL RESEARCH

Effect of magnetic nanoparticles of Fe$_3$O$_4$ and wogonin on the reversal of multidrug resistance in K562/A02 cell line

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Background: Multidrug resistance is the main obstacle to the efficiency of systemic chemotherapy against hematologic malignancy. This study investigated the reversible effect of the copolymer wogonin and daunorubicin coloaded into Fe$_3$O$_4$ magnetic nanoparticles, and the mechanism potentially involved.

Methods: The growth inhibition rate of K562/A02 cells was investigated by MTT assay, and apoptosis of cells and the intracellular daunorubicin concentration were detected by flow cytometry. Distribution of nanoparticles taken up by K562/A02 cells was observed under a transmission electron microscope and demonstrated by Prussian blue staining. The transcription level of MDR1 mRNA and expression of P-glycoprotein were determined by reverse transcriptase polymerase chain reaction and Western blotting assay, respectively.

Results: The reversible effect of daunorubicin-wogonin magnetic nanoparticles was 8.87-fold that of daunorubicin + wogonin and of daunorubicin magnetic nanoparticles. Transmission electron microscopy and Prussian blue staining revealed that the nanoparticles were located in the endosome vesicles of cytoplasm. Also, the apoptosis rate and accumulation of intracellular daunorubicin in the daunorubicin-wogonin magnetic nanoparticle group were significantly higher than that in the daunorubicin, daunorubicin + wogonin, and daunorubicin magnetic nanoparticle groups. Furthermore, transcription of MDR1 mRNA and expression of P-glycoprotein in K562/A02 cells were significantly downregulated in the daunorubicin-wogonin magnetic nanoparticle group compared with the other groups.

Conclusion: These findings suggest that the remarkable effects of the novel daunorubicin-wogonin magnetic nanoparticle formulation on multidrug resistant K562/A02 leukemia cells would be a promising strategy for overcoming multidrug resistance.

Keywords: magnetic nanoparticles, Fe$_3$O$_4$, wogonin, multidrug resistance, daunorubicin, P-glycoprotein

Introduction

Multidrug resistance is the major obstacle to the efficiency of chemotherapy in the treatment of leukemia.[1] The mechanisms associated with multidrug resistance in cancer have been widely explored, and chemotherapy-induced upregulation of P-glycoprotein is considered the major event in establishing multidrug resistance in cancer cells.[2] Much research attention has been focused on the discovery and development of agents that can inhibit P-glycoprotein with high efficiency and low toxicity.[3–5] However, these compounds, with their low efficiency and/or high toxicity, are often nonspecific.[6,7] The first and second generations of P-glycoprotein inhibitors have now been tested in clinic trials, but their therapeutic effects and safety profiles have not been ideal.[6,7] Successful management of cancers with overexpressed P-glycoprotein would be greatly aided

by novel agents with high efficiency and/or low toxicity. Wogonin (5,7-dihydroxy-8-methoxy flavone) is a flavone originating from the roots of Scutellaria baicalensis Georgi. One study has shown that wogonin 10–30 µmol/L acted as an inhibitor of P-glycoprotein and consequently increased the cellular content of chemotherapeutic agents in multidrug resistant cancer cells.[8] On the other hand, wogonin can inhibit apoptosis induced by chemotherapeutic agents in normal cells, such as thymocytes.[9]

However, sequential or concurrent administration of a chemosensitizer and a cytotoxic drug or a combination of drugs cannot guarantee the co-action of intended drugs in the same cancer cells because of their different pharmacokinetics and tissue disposition. It is exciting that magnetic nanoparticles, with their biodegradable nature, biocompatibility, and low toxicity, possess the capability to encapsulate a single drug or multiple drugs with a variety of properties, ranging from highly water-soluble to poorly water-soluble.[10–13] In addition, its passive targeting properties may reduce side effects during chemotherapy,[14] rendering it a promising drug delivery system.

Therefore, in this study, to overcome the dose-limiting side effects of conventional chemotherapeutic agents, as well as to reduce the risk of therapeutic failure as a result of multidrug resistance, we undertook a rational design of biocompatible magnetic nanoparticles for sustained delivery of wogonin and daunorubicin and also investigated the potential mechanisms involved.

Materials and methods
Chemicals
Daunorubicin hydrochloride (Huifengda Chemical Co, Jinan, China), wogonin (Key Laboratory of Carcinogenesis and Intervention, Jiangsu Province, China Pharmaceutical University, China), RPMI 1640 medium (Gibco/BRL, Gaithersburg, MD), newborn bovine serum (Sijiqing, Hangzhou, China), adriamycin (Hisun Phamaceutical Co, Zhejiang, China),TRIzol® (Invitrogen, Carlsbad, CA) were used. A reverse transcriptase polymerase chain reaction (RT-PCR) kit was purchased from Takara Biotechnology (Dalian, China). Monoclonal antibodies of P-glycoprotein and β-actin were from Santa Cruz Biotechnology (Santa Cruz, CA). All other chemicals were of analytic grade.

Preparation of drug-loaded Fe_3O_4 magnetic nanoparticles
Wogonin was dissolved in absolute dimethyl sulfoxide to give a 1 mol/L solution, and the final concentration of dimethyl sulfoxide in the medium of wogonin-treated cells was less than 0.05% (v/v), showing no toxicity in K562/A02 cells.[15] Magnetic nanoparticles were produced using the electrochemical deposition method[13] for use in the present experiment in order to obtain a colloidal suspension of magnetic nanoparticles.

In total, 10 µmol of magnetic nanoparticles were well distributed by ultrasound in 10 mL of RPMI 1640 medium containing 10% (v/v) inactivated newborn bovine serum.[16] Daunorubicin was dissolved in RPMI 1640 medium containing 10% (v/v) inactivated newborn bovine serum to obtain a 1 mmol/L solution. Various concentrations of daunorubicin and wogonin 20 µmol/L were added into the aqueous dispersion of magnetic nanoparticles at a molar ratio of 1/2/4/8/16/32/64:20:100. The mixture was kept below 4°C for 48 hours to enable the drugs to conjugate with the magnetic nanoparticles by mechanical absorption polymerization.[10,13]

Cell culture
K562, a human chronic myeloid leukemia blast crisis cell line, and K562/A02, a K562 cell line resistant to adriamycin, were cultured in RPMI 1640 medium supplemented with 10% newborn bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37°C in a humidified atmosphere containing 5% CO_2, and passaged every two days. The resistant cell line was incubated in the presence of adriamycin 1 µg/mL until at least 3 days before starting the experiments.

Cytotoxicity assay
In vitro cytotoxicity was measured using the MTT assay. K562/A02 cells were seeded in 96-well plates at a density of 2×10^3 cells/well per 0.1 mL of medium, and incubated with daunorubicin 1–64 µmol/L, Fe_3O_4 magnetic nanoparticles 0.1–0.5 (v/v), wogonin 10–50 µmol/L, daunorubicin 1–64 µmol/L + wogonin 20 µmol/L, daunorubicin magnetic nanoparticles at a molar ratio of 1/2/4/8/16/32/64:100, or daunorubicin-wogonin magnetic nanoparticles at a molar ratio of 1/2/4/8/16/32/64:20:100 at 37°C for 24, 48, and 72 hours. Meanwhile, RPMI 1640 medium was used as the blank control. After incubation, 20 µL of MTT 0.5 mg/mL were added to each well and cultured for 4 hours at 37°C. Thereafter, 150 µL of dimethyl sulfoxide were added to each well to dissolve the formazan crystals using an automated shaker to stir the cells slightly. Absorbance of the suspension was measured by optical density (OD) at a wave length of 490 nm. The cell inhibition ratio (%) was calculated as (1 − OD treated group/OD control group) × 100. The IC_{50}

was defined as the concentration required for 50% inhibition of cell growth.

Qualitative and quantitative evaluation of Fe$_3$O$_4$ magnetic nanoparticle uptake in cells

Transmission electron microscopy

K562/A02 cell suspensions were seeded at a density of 5×10^5 cells/well in 6-well plates and incubated with 10% (v/v) magnetic nanoparticles for 48 hours at 37°C. After incubation, the cells were collected and fixed for 4 hours at 4°C in 2.5% glutaraldehyde, and fully washed in 0.1 mol/L phosphate-buffered saline three times. The cells were then post-fixed at 4°C in 2% osmium tetroxide for 2 hours, and dehydrated in a graded series of ethanols and embedded in epoxy resin. Thereafter, the embedded cells were cut into ultrathin sections (75 nm) and stained with uranyl acetate and lead citrate. Finally, the sections were viewed by transmission electron microscopy (JEM-2100, JEOL, Tokyo, Japan).

Optical microscopy

After incubating with 0.1 (v/v) magnetic nanoparticles for 48 hours at 37°C, the cells were harvested and made into cell smears. Before observation by optical microscopy, the cells were stained with Prussian blue. The cells on the slide were continuously incubated for 30 minutes in 2% potassium ferrocyanide and 6% hydrochloric acid, and then counterstained with nuclear fast red for 3 minutes. The smears were viewed under an optical microscope (B × 41M, Olympus, Tokyo, Japan) at 1000 × amplification.

Apoptosis assay by flow cytometry

After incubation with different drugs, the cells were collected at 48 hours, washed twice with phosphate-buffered saline, and suspended in 200 µL of binding buffer and 10 µL of Annexin V-FITC for 20 minutes in the dark. Analyses were done using a FACSCalibur® flow cytometer (Becton Dickinson, San Antonio, TX) with Cell Quest™ software.

Cellular accumulation of daunorubicin

Cellular accumulation of daunorubicin was analyzed by flow cytometry. In brief, after incubation with different drugs for 48 hours, the cells were collected and washed with 0.1 mol/L phosphate-buffered saline three times. Thereafter, 500 µL of phosphate-buffered saline was added to each sample to resuspend the cells. The cellular accumulation of daunorubicin in each sample was determined using the flow cytometer at a wave length of 488 nm.

RT-PCR assay

The RT-PCR method was used to evaluate the qualitative efficacy of MDR1 with drugs as previously described at the transcription level. After incubation, the cells were lysed and total RNA was extracted with TRIzol. 4 µg of total RNA was added to reverse transcriptase buffer, 25 mmol/L of MgCl$_2$, 10 mmol/L deoxyribonucleotide triphosphates, random 9 mers (50 pmol/µL), RNase inhibitor (40 U/µL), and avian myeloblastosis virus reverse transcriptase (5 U/µL) to provide a final total volume of 25 µL. To obtain cDNA, the conditions of reverse transcriptase were 42°C for one hour, 85°C for 5 minutes, and then 5°C for 5 minutes. The designed PCR primers included MDR1 primer (sense primer 5′-AACGGAAGCCAGAACATTCC-3′, antisense primer 5′-AGGCTTCCTGTGGCAAAGAG-3′) and β-actin primer (sense primer 5′-GCTCGTCGT CGACAACG GCTC-3′, anti-sense primer 5′-CAAACATGATCTGGGT CATCTTCTC-3′). The amplified PCR products were 353 base pairs for β-actin and 180 base pairs for MDR1. The newly synthesized cDNA was amplified by PCR, each cycle comprising denaturation at 95°C for 40 seconds, annealing at 52°C for 30 seconds, and elongation at 72°C for 35 seconds. Predenaturing was performed at 95°C for 2 minutes and final extension at 72°C for 10 minutes. RT-PCR products were analyzed by ScnImage software (Scion Corporation, Frederick, MD) with ethidium bromide-stained 1.5% agarose gels.

Western blotting assay

After treatment as before, total protein was isolated on ice and subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis using a modified radioimmunoprecipitation assay buffer, and then transferred to a polyvinylidene difluoride membrane (65421, Pall Corporation, Port Washington, NY). Nonspecific binding sites were blocked with 5% nonfat milk for one hour at room temperature. The blots were stained with mouse monoclonal antihuman P-glycoprotein (1:200) or β-actin (1:400) antibodies overnight at 4°C, and then followed by horseradish peroxidase-labeled rabbit-mouse immunoglobulin G (1:5000) as a secondary antibody. The blots were visualized by enhanced chemiluminescence (ECL system, Amersham, UK), and β-actin was used as the internal control.

Statistical analysis

All data were expressed as the mean ± standard deviation, and analyzed using SPSS software (version 18.0, SPSS Inc, Chicago, IL). Differences between the groups were evaluated using one-way analysis of variance. A value of $P < 0.05$ was considered to be statistically significant.
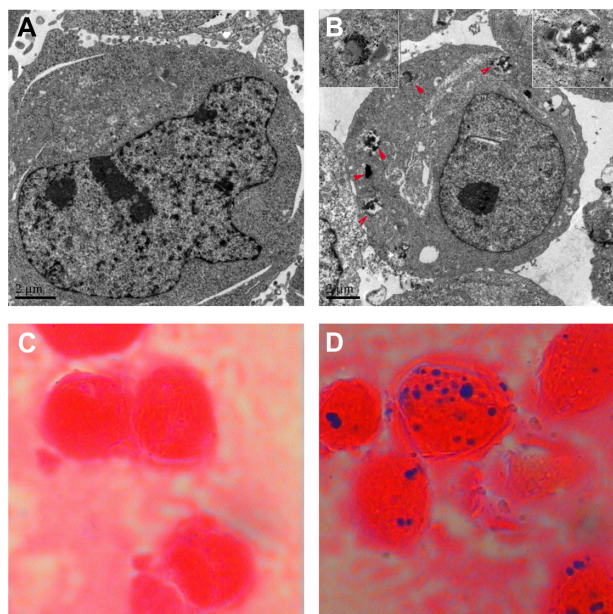
Figure 1 Distribution of nanoparticles in K562/A02 cells incubated with and without 10% (v/v) magnetic nanoparticles for 48 hours.
Notes: (**A**) Transmission electron micrograph for control, (**B**) transmission electron micrograph for 10% (v/v) magnetic nanoparticles, red arrows direct nanoparticles in the endosome vesicles, and the upper left and upper right pictures were the magnifications (10×), (**C**) Prussian blue staining for control, and (**D**) Prussian blue staining for 10% (v/v) magnetic nanoparticles.

Results

Nanoparticles located in the endosome vesicles of the cytoplasm were observed by transmission electron microscopy, and nearly 100% of the cells were labeled with Prussian blue stain after incubation for 48 hours. The diameter of a single blank magnetic nanoparticle was 16.72 ± 1.37 nm.

Cell survival

As the results of the MTT assay show, daunorubicin, a combination of daunorubicin + wogonin, daunorubicin magnetic nanoparticles, and daunorubicin-wogonin magnetic nanoparticles showed antiproliferative activity against drug-resistant cells in a dose and time-dependent manner (Figure 2D–F). Meanwhile, 0.1–0.4 (v/v) magnetic nanoparticles alone and 0–40 µmol/L wogonin alone did not have a significant influence on cell proliferation (survival faction > 90%,[10] Figure 2A and B), and neither 20 µmol/L wogonin nor 0.1 (v:v) magnetic nanoparticles induced significant cell inhibition at 24, 48, and 72 hours (Figure 2C). After incubation for 48 hours, the reversible effect of daunorubicin-wogonin magnetic nanoparticles ($IC_{50\ daunorubicin}/IC_{50\ daunorubicin-wogonin\ magnetic\ nanoparticles}$) was 8.87-fold, which was higher than that of daunorubicin + wogonin ($IC_{50\ daunorubicin}/IC_{50\ daunorubicin\ +\ wogonin}$, 4.85-fold) and daunorubicin-magnetic

nanoparticles ($IC_{50\ daunorubicin}/IC_{50\ daunorubicin-magnetic\ nanoparticles}$, 3.9-fold). The difference was statistically significant ($P < 0.05$).

Apoptosis assay by flow cytometry

After incubation for 48 hours, the apoptotic rates of K562/A02 cells treated with the control, daunorubicin, magnetic nanoparticles, wogonin, daunorubicin + wogonin, daunorubicin magnetic nanoparticles, and daunorubicin-wogonin magnetic nanoparticles were $7.80\% \pm 0.36\%$, $9.08\% \pm 0.33\%$, $8.23\% \pm 0.47\%$, $8.71\% \pm 0.54\%$, $33.65\% \pm 1.96\%$, $28.47\% \pm 2.28\%$, and $41.04\% \pm 2.63\%$, respectively. Compared with the control group, apoptotic rates in the daunorubicin + wogonin, daunorubicin magnetic nanoparticle, and daunorubicin-wogonin magnetic nanoparticle groups were significantly increased ($P < 0.05$). Although the combination of daunorubicin + wogonin induced significant apoptosis, daunorubicin-wogonin magnetic nanoparticles showed much higher induction of apoptosis ($P < 0.05$, Figure 3).

Cellular accumulation of daunorubicin

The relative fluorescence intensity (fluorescence intensity-treated group/fluorescence intensity control group) was 3.74 ± 0.34 for K562/A02 cells incubated with 2 µmol/L of daunorubicin for 48 hours, 14.71 ± 0.84 for daunorubicin + wogonin, 12.71 ± 0.65 for daunorubicin magnetic nanoparticles, and 24.31 ± 2.82 for daunorubicin-wogonin magnetic nanoparticles. The daunorubicin + wogonin, daunorubicin magnetic nanoparticle, and daunorubicin-wogonin magnetic nanoparticle groups showed higher daunorubicin accumulation than did the daunorubicin alone group ($P < 0.05$). Notably, daunorubicin-wogonin magnetic nanoparticles led to an increase in intracellular daunorubicin concentration compared with the daunorubicin + wogonin and daunorubicin magnetic nanoparticle groups ($P < 0.05$).

Expression of MDR1/P-glycoprotein in K562/A02 cells

MDR1 mRNA was not detected in drug-sensitive K562 cells (data not shown), and was overexpressed in drug-resistant K562/A02 cell lines. Both daunorubicin and wogonin groups can downregulate transcription of MDR1 mRNA to some extent, and both were strengthened by the addition of magnetic nanoparticles. Compared with the control group, MDR1 mRNA transcriptions were significantly inhibited by nearly $44.85\% \pm 3.89\%$ in the wogonin group, by $59.13\% \pm 3.48\%$ in the daunorubicin + wogonin group, and by $75.80\% \pm 4.32\%$ in the daunorubicin-wogonin magnetic nanoparticle group ($P < 0.05$, Figure 5). The expression of
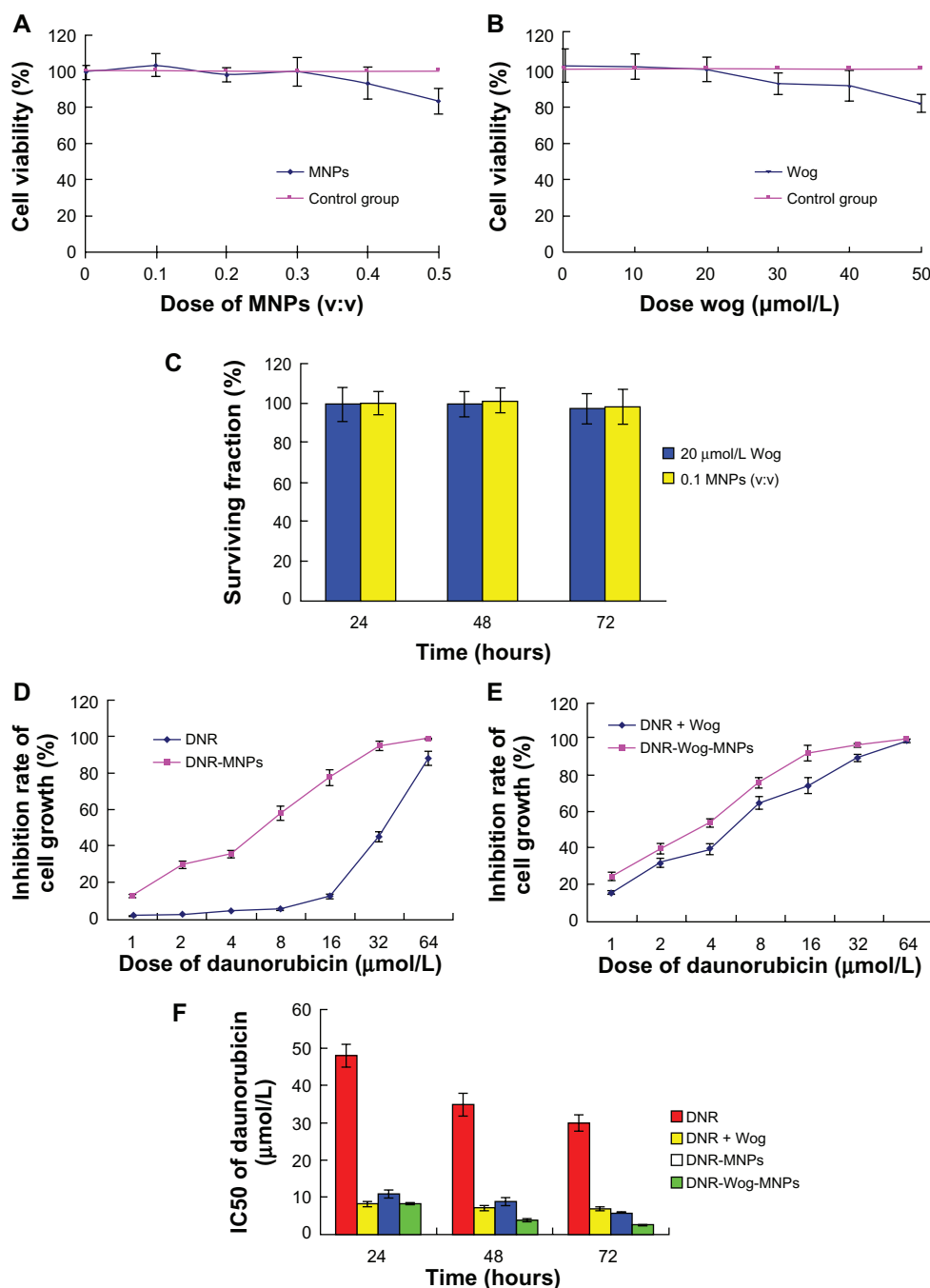
Figure 2 (**A**) Viability of K562/A02 cells treated with different concentrations of magnetic nanoparticles, (**B**) viability of K562/A02 cells treated with different concentrations of wogonin, (**C**) surviving fraction of K562/A02 cells treated with 20 μmol/L wogonin or 0.1 (v/v) magnetic nanoparticles for 24, 48, and 72 hours, (**D**) inhibition rates of K562/A02 cells treated with different concentrations of daunorubicin with and without magnetic nanoparticles for 48 hours, (**E**) inhibition rates of K562/A02 cells treated with coadministered daunorubicin + wogonin with and without magnetic nanoparticles for 48 hours, (**F**) IC$_{50}$ of daunorubicin treated with daunorubicin, daunorubicin + wogonin, daunorubicin magnetic nanoparticles, and daunorubicin-wogonin magnetic nanoparticles for 24, 48, and 72 hours.
Abbreviations: DNR, daunorubicin; Wog, wogonin; MNPs, magnetic nanoparticles; IC$_{50}$, inhibitory concentration at 50%.

P-glycoprotein were significantly downregulated by nearly 40.62% ± 2.57% in the wogonin group, by 69.93% ± 4.63% in the daunorubicin + wogonin group, and by 79.51% ± 4.48% in the daunorubicin-wogonin magnetic nanoparticle group, respectively, when compared with the control group ($P < 0.05$, Figure 6).

Discussion

A novel copolymer containing a nanoparticle, chemotherapeutic agent, and chemosensitizer with high efficiency and high potency has been devised in this work. Although there have been reports of sequential or concurrent administration of cytotoxic drugs and P-glycoprotein inhibitors, none could
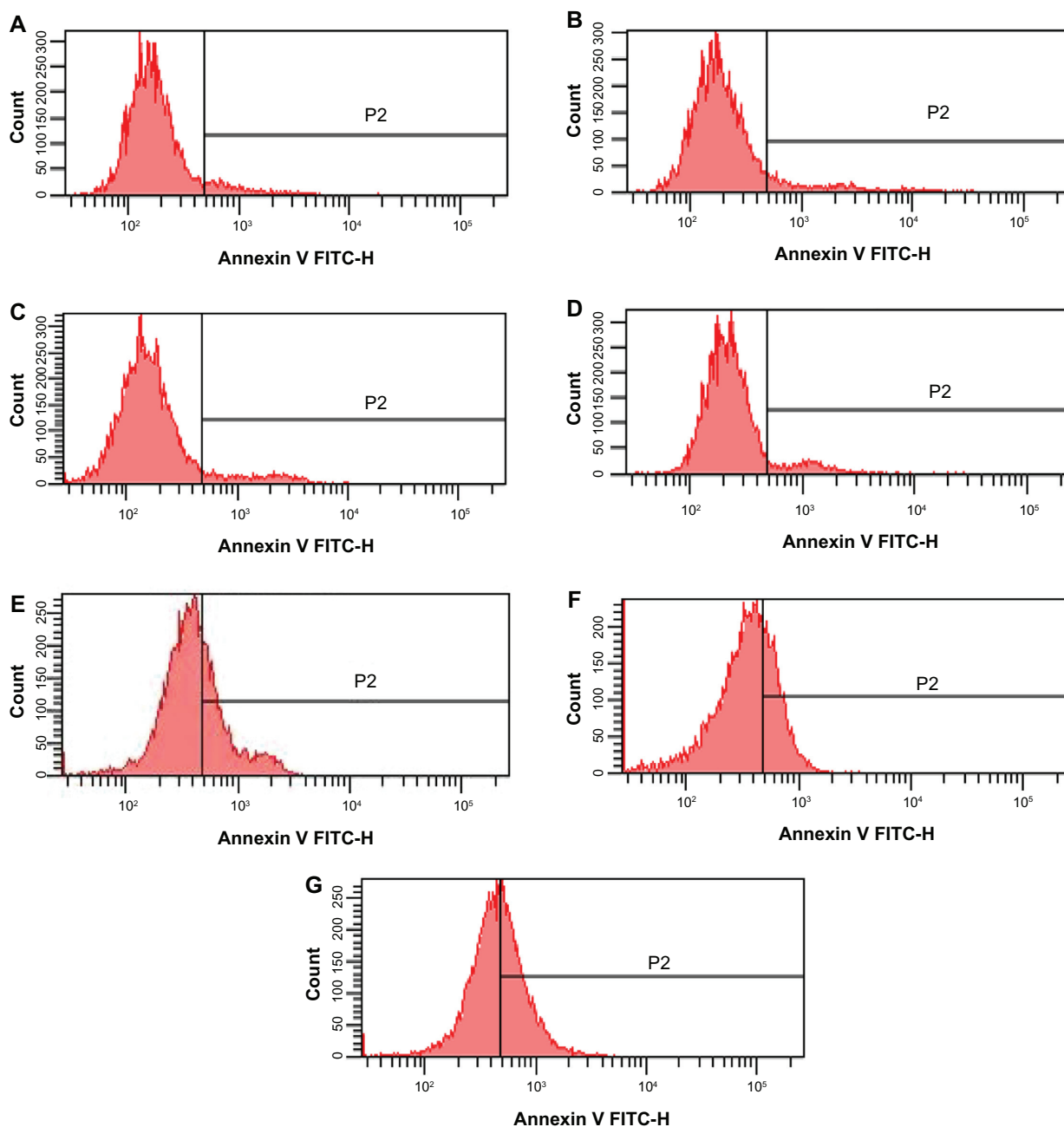
Figure 3 Apoptotic rates of K562/A02 cells incubated for 48 hours by flow cytometry. (**A**) control, (**B**) 2 μmol/L daunorubicin, (**C**) 0.1 (v/v) magnetic nanoparticles, (**D**) 20 μmol/L wogonin, (**E**) combination of 2 μmol/L daunorubicin and 20 μmol/L wogonin, (**F**) 2μmol/L daunorubicin magnetic nanoparticles (at a molar ratio of 2:100), (**G**) 2 μmol/L daunorubicin-wogonin magnetic nanoparticles (at a molar ratio of 2:20:100).

guarantee that the two drugs will have activity in the same cancer cells due to their different pharmacokinetics and tissue disposition. Since over expression of P-glycoprotein is the main cause of multidrug resistance in leukemia systemic chemotherapy,[2] we choose this combination.

Daunorubicin is an anthracycline and a substrate for P-glycoprotein,[17] and the current standard of care for induction chemotherapy of acute myeloid leukemia includes 3 days of daunorubicin.[18] Although intensification of the

dose of daunorubicin (90 mg/m^{2}) improves the complete remission rate in acute myeloid leukemia,[19,20] it will result in hematologic toxicity or adverse events.[18] A low dose exposes some populations of tumor cells to sublethal doses of the chemotherapeutic agent used, resulting in onset of the multidrug-resistant phenotype.[21] If one agent having the same or higher cytotoxicity can lower the dose of drug we utilize nowadays, it might be a promising solution for multidrug resistance. The copolymer in our research can
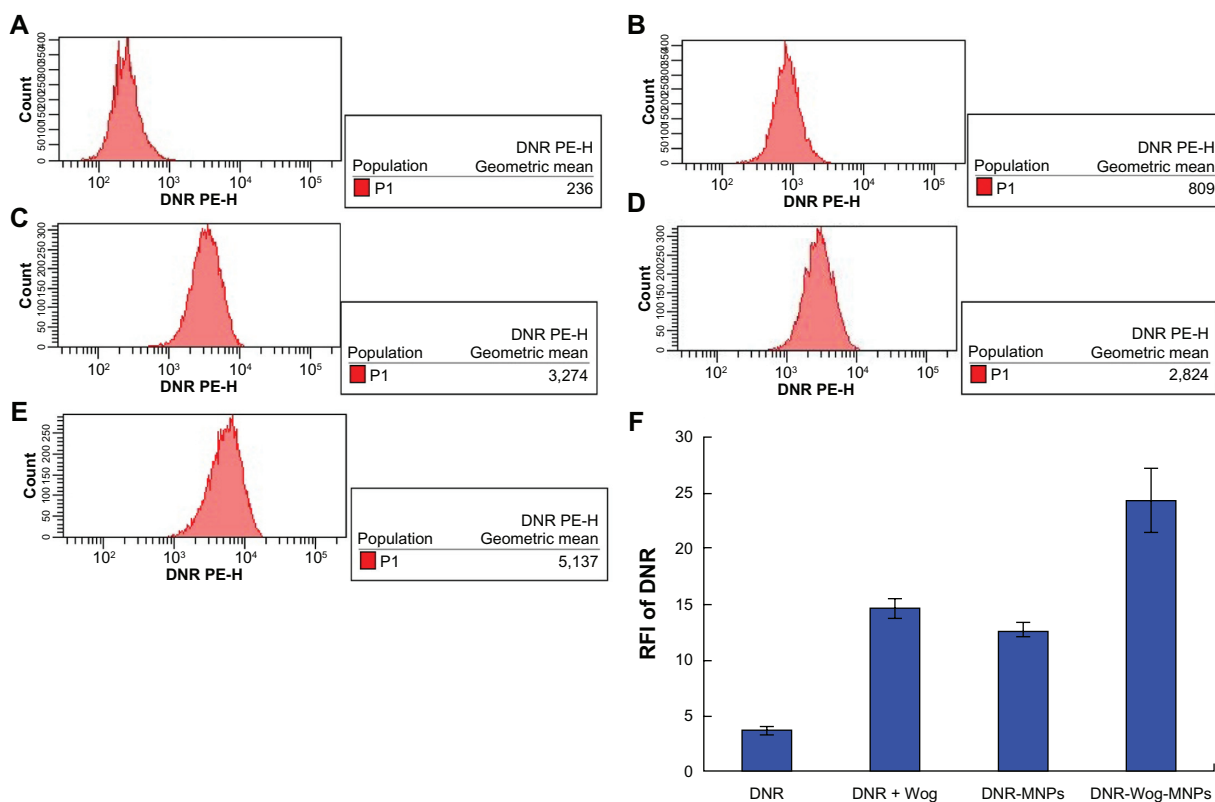
Figure 4 Intracellular accumulation of daunorubicin in K562/A02 cells cultured with different drugs for 48 hours. (**A**) Control, (**B**) 2 μmol/L daunorubicin, (**C**) combination of 2 μmol/L daunorubicin combined with 20 μmol/L Wog, (**D**) 2 μmol/L daunorubicin magnetic nanoparticles (at a molar ratio of 2:100), (**E**) 2 μmol/L daunorubicin-Wog magnetic nanoparticles (at a molar ratio of 2:20:100), (**F**) relative fluorescence intensity of daunorubicin in treated cells.
Abbreviations: DNR, daunorubicin; Wog, wogonin; MNPs, magnetic nanoparticles; RFI, relative fluorescence intensity.

lower the dose of daunorubicin used by 8.87-fold and owned higher fluorescence of daunorubicin compared with daunorubicin used alone. Even daunorubicin loaded with magnetic nanoparticles can lower the dose of daunorubicin needed by 3.9-fold, its reversal power was significantly lower than that of daunorubicin-wogonin magnetic nanoparticles.

Wogonin, a flavone originating from the root of a Chinese herb, *Scutellaria baicalensis* Georgi, can impair the function of P-glycoprotein and increase the cellular content of the chemotherapeutic agent entering multidrug-resistant cells.[8] In addition, the inhibitory potency of wogonin was nearly equal to that of the first-generation P-glycoprotein inhibitor,[3] verapamil.[8] We had taken the lead to investigate the influence of wogonin, and the results show that it did downregulate transcription of MDR1 mRNA and expression of P-glycoprotein in K562/A02 cells. Because this flavone does not generate significant cytotoxicity, increase apoptosis in multidrug-resistant cancer cells, or decrease induction of apoptosis in normal cells,[9] wogonin could be an ideal P-glycoprotein inhibitor with high efficiency and low toxicity.

Magnetic nanoparticles, a promising biocompatible material, have the features of satisfactory water solubilization, biocompatibility, and easy functionalization.[10] A previous study in our laboratory showed that magnetic nanoparticles could improve the sensitivity of anticancer drugs and increase their effectiveness.[12,13] We also found that magnetic nanoparticles have the capability to load single or multiple drugs with a variety of properties.[11,12] The hydrodynamic diameter of ideal nanoparticles ranges from 10 nm to 100 nm, as reported.[22] If the diameter is less than 10 nm, most of them will undergo extravasation in tissue and be cleared by the kidney.[23] However, if the diameter exceeds 100 nm, the nanoparticles will soon be eliminated from the circulation by the reticuloendothelial system.[23] In the present study, the mean size of the blank magnetic nanoparticles was 16.72 ± 1.37 nm, indicating that citric acid-functionalized magnetic nanoparticles were suitable for biological application and drug delivery. When the concentration of magnetic nanoparticles was 0.1 (v/v), they showed no obvious toxicity (Figure 2C) or apoptosis (Figure 3) to K562/A02 cells. Transmission electron microscopy suggested that the magnetic nanoparticles are taken up by the cell via membrane-bound vesicles, and shuttled to the
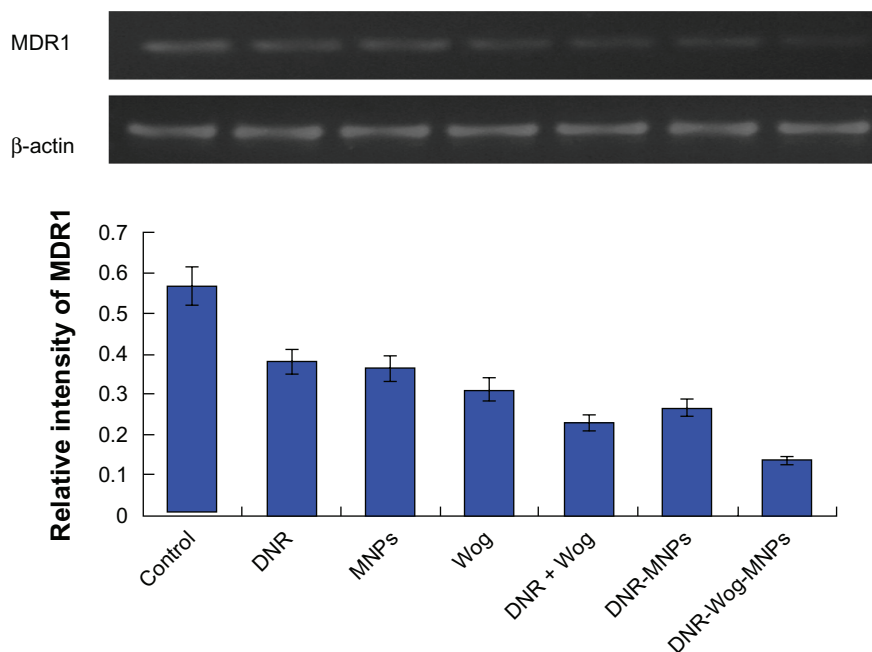
Figure 5 Transcription of MDR1 mRNA after treatment for 48 hours.
Abbreviations: DNR, daunorubicin; MNPs, magnetic nanoparticles; Wog, wogonin.

cytosol in K562/A02 cells. The magnetic nanoparticles were stained using potassium ferrocyanide (blue), while the cells were stained using nuclear fast red. There were no blue granules in the controls; on the contrary, nearly 100% of multidrug resistant cells were labeled. Thus, the ability to overcome efflux pumps in the cell membrane and transport an active drug into the cell might be one of the proposed mechanisms that allows magnetic nanoparticles to be a potential strategy to overcome multidrug resistance.

Although inhibitors of P-glycoprotein have been developed as a way to overcome multidrug resistance, rapid drug metabolism may be one of the factors influencing the effect of treatment.[24] It should be noticed that there were no significant differences in IC_{50} of daunorubicin between
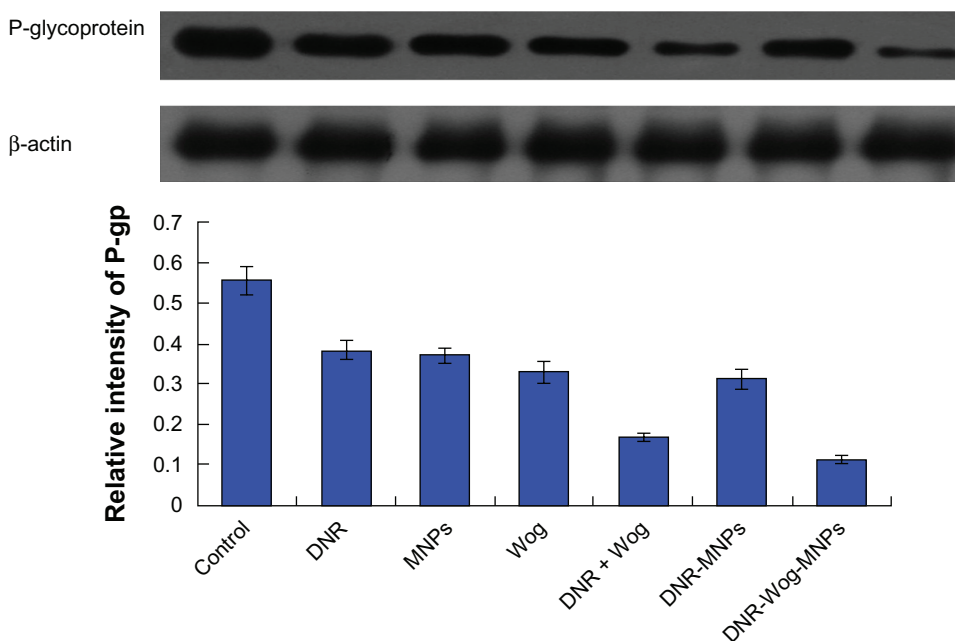

Figure 6 Expression of P-glycoprotein after treatment for 48 hours.
Abbreviations: DNR, daunorubicin; MNPs, magnetic nanoparticles; Wog, wogonin.

the daunorubicin + wogonin and daunorubicin-wogonin magnetic nanoparticle groups at 24 hours, but this situation changed at 48 hours and 72 hours, when the IC_{50} of daunorubicin in the daunorubicin-wogonin magnetic nanoparticle group was obviously lower than that in the daunorubicin + wogonin group. Possible reasons for this phenomenon might be that a large proportion of wogonin in the daunorubicin + wogonin group may have been metabolized to a residue which could inhibit P-glycoprotein further, and there is some evidence in the literature to support this,[25] and the sustained-release properties of daunorubicin-wogonin magnetic nanoparticles might enable continuous release of the chemosensitizing agent, instead of rapid metabolism, and much more daunorubicin was released continuously from the copolymer after 24 hours. Also, a previous study in our laboratory showed that drug-loaded magnetic nanoparticles were able to release daunorubicin in a sustained manner for 25 days, and less than 20% of daunorubicin was released from the magnetic nanoparticles in 24 hours.[11] This sustained release might lead to an effective dose between two cycles of chemotherapy with maximal killing of malignant cells.

A copolymer with the power to reverse multidrug resistance might enable particles to be taken up by membrane-bound vesicles into cells such that their cargo becomes distal to the cell membrane and is inaccessible to the effects of ABC transporter-mediated drug efflux (Figure 1), raise the daunorubicin level in multidrug-resistant cells by releasing the copolymer from the vesicles (Figure 4), and downregulate expression of P-glycoprotein (Figure 6) at the same time. We believe that downregulation of P-glycoprotein and passive targeting of nanoparticles raise the intracellular concentration of daunorubicin, and the copolymer of daunorubicin-wogonin magnetic nanoparticles can induce the cell apoptosis rate to a higher degree as a result. We did not investigate sequential or concurrent administration of separate P-glycoprotein inhibitors and anticancer drug-loaded nanoparticles, but there have been reports demonstrating chemosensitizer coloading with an anticancer drug in the same nanoparticle resulting in higher drug uptake than found with coadministration of nanoparticles loaded with a single agent.[26,27]

Conclusion

These results indicate that the unique properties of daunorubicin-wogonin magnetic nanoparticles can reverse multidrug resistance in K562/A02 cells, which would be a promising strategy for overcoming multidrug resistance in the future.

Disclosure

The authors report no conflicts of interest in this work.

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