

Daunorubicin-loaded magnetic nanoparticles of Fe_3O_4 overcome multidrug resistance and induce apoptosis of K562-n/VCR cells *in vivo*

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Abstract: Multidrug resistance (MDR) is a major obstacle to cancer chemotherapy. We evaluated the effect of daunorubicin (DNR)-loaded magnetic nanoparticles of Fe_3O_4 (MNPs- Fe_3O_4) on K562-n/VCR cells *in vivo*. K562-n and its MDR counterpart K562-n/VCR cell were inoculated into nude mice subcutaneously. The mice were randomly divided into four groups: group A received normal saline, group B received DNR, group C received MNPs- Fe_3O_4 , and group D received DNR-loaded MNPs- Fe_3O_4 . For K562-n/VCR tumor, the weight was markedly lower in group D than that in groups A, B, and C. The transcriptions of Mdr-1 and Bcl-2 gene were significantly lower in group D than those in groups A, B, and C. The expression of Bcl-2 was lower in group D than those in groups A, B, and C, but there was no difference in the expression of P-glycoprotein. The transcriptions and expressions of Bax and caspase-3 in group D were increased significantly when compared with groups A, B, and C. In conclusion, DNR-loaded MNPs- Fe_3O_4 can overcome MDR *in vivo*.

Keywords: multidrug-resistance reversal, leukemia, magnetic nanoparticles of Fe_3O_4 , *in vivo*

Introduction

The development of new cytotoxic drugs and treatment strategies has resulted in improved response rates for patients with hematological malignancies. However, multidrug resistance (MDR) is the primary cause for almost 90% of cancer treatment failures.^{1,2} Cancer cells are becoming resistant to the cytotoxic effects of a wide range of structurally and mechanistically unrelated anticancer drugs. It has been proved that various molecular mechanisms,³ including the activation of detoxifying systems of the glutathione-S-transferase gene, DNA repair, and alteration in drug-induced apoptosis of genes in the Bcl-2 pathway, contribute to the complex story of cancer drug resistance. However, the most commonly encountered effect of MDR in the laboratory is the decreased intracellular drug accumulation caused by enhanced drug efflux from tumor cells.⁴ This is related to the overexpression of a family of energy-dependent transporters,¹ known as ATP-binding cassette (ABC) transporters such as P-glycoprotein (P-gp) and MDR-associated protein.^{2,5} Among these ABC transporters, P-gp confers cancer cells the strongest resistance to the widest variety of compounds. P-gp, a 170-kDa membrane-associated glycoprotein, transports a broad class of hydrophobic cytotoxic drugs that are central to most chemotherapeutic regimens from cell cytoplasm to outside the plasma membrane.⁶ Despite the wealth of information collected about the biochemistry and substrate specificity of P-gp and other types of ABC transporters, translation of this knowledge from the bench to the bedside has proved to be unexpectedly difficult. Because cytotoxic drugs typically

carry numerous dose-limiting normal tissue side effects,⁷ it is generally impractical to overcome this form of drug resistance simply by increasing the drug dose. Extensive efforts have been made to design P-gp modulators (chemosensitizers). Unfortunately, modulation of P-gp activity by current chemosensitizers to inhibit ABC transporters is limited for several reasons, such as low efficiency, strong adverse effects, and pharmacokinetic interactions that limit anticancer drug clearance and metabolism.²

Nanotechnology is no stranger to oncology.⁸ A wide variety of nanovectors have been used for the delivery of anticancer drugs to overcome drug resistance as well as to improve the effectiveness and safety of cancer chemotherapy,⁹ such as the use of drug-loaded liposomes,^{10–12} stealth liposomes,^{13,14} polymeric nanospheres with equivalent stealth properties, polymeric nanocapsules,^{15,16} and solid lipid nanoparticles.¹⁷ The major advantages sought by the use of nanovectors over simple drugs are as follow: the specific delivery of large amounts of therapeutic agents using biorecognition targets;¹⁸ protection of the drug from premature degradation and interaction with the biological environment; enhanced absorption of the drugs into a selected tissue through enhanced permeability and retention (EPR) effect; controlling the pharmacokinetic and drug tissue distribution profile; and improvement of intracellular penetration,¹⁹ which ameliorates the therapeutic index.

In our previous research, we have developed tetraheptylammonium-capped MNPs-Fe₃O₄,^{20,21} which could facilitate the drug accumulation of daunorubicin (DNR) inside MDR leukemia K562/A02 cells and enhance the response of DNR in MDR leukemia K562/A02 cells *in vitro*.²¹ It has been demonstrated that DNR polymerized with MNPs-Fe₃O₄ have shown more chemosensitizing activities than those of DNR alone. The cytotoxicity test *in vitro* revealed that MNPs-Fe₃O₄ exhibit excellent biocompatibility.²² Furthermore, no carcinogenic effects have been observed for MNPs-Fe₃O₄.²³ Its effect on MDR leukemic cells *in vivo* remains unknown. In this work, we demonstrate that DNR-loaded MNPs-Fe₃O₄ are able to reverse MDR leukemic K562-n/VCR cells *in vivo* by inducing apoptosis. However, DNR-loaded MNPs-Fe₃O₄ fails to enhance cytotoxicity response in sensitive leukemic K562-n cells *in vivo*.

Materials and methods

Preparation of DNR-loaded MNPs-Fe₃O₄

MNPs-Fe₃O₄ were produced by electrochemical deposition under oxidizing conditions in a 0.1 mol/L tetraheptylammonium 2-propanol solution, where the magnetization and

the size of MNPs-Fe₃O₄ was found to be 25.6×10^{-3} emu/mg and 30 nm, respectively. The deposited clusters were capped with tetraheptylammonium, which acts as a stabilizer of the colloidal nanocrystallites.²⁰ Before being applied in this experiment, the magnetized nanoparticles of Fe₃O₄ were well distributed in 0.9% NaCl solution by using ultrasound treatment in order to obtain colloidal suspension of MNPs-Fe₃O₄. 0.2 g/L DNR (Sigma Aldrich, St. Louis, MO, USA) conjugated with 0.116 g/L MNPs-Fe₃O₄ were prepared by mechanical absorption polymerization as previously reported.^{24,25}

Cell lines and cell culture

K562-n, a cell strain with high tumorigenicity in nude mice, was derived from K562 cells, which were cloned from human chronic myelogenous leukemia by repeated passage in nude mice and in culture alternatively.²⁶ MDR leukemia cell K562-n/VCR, which expresses MDR gene (*mdr-1*), was established by a long-term, intermittent, low-dose, and gradually escalated vincristine (VCR) added into the culture medium. K562-n/VCR has been proved to be resistant to some extent to many cytotoxins including VCR, DNR, and has high tumorigenicity in nude mice.²⁷ It has been proved that K562-n/VCR cells in the xenograft model had a comparatively stable MDR phenotype to the corresponding cells.²⁸ K562-n and K562-n/VCR cells (gifted from the department of hematology, Shanghai Hospital, Second Military Medical University, Shanghai, China) were maintained in RPMI 1640 medium (Gibco, Carlsbad, CA, USA) with 10% fetal bovine serum (Sijiqing, Hang Zhou, China), penicillin G (100 IU/mL), and streptomycin (100 µg/mL) at 37 °C in a humidified 5% CO₂ atmosphere. 0.64 µg/ml VCR was added regularly in K562-n/VCR culture medium to maintain drug resistance. K562-n/VCR cells were incubated in VCR-free medium for over two weeks before the planned experiment.

Animals and establishment of the xenograft leukemia model in nude mice

Female BALB/c-nu/nu mice were purchased from Beijing National Center for Laboratory Animals, the Chinese Academy of Medical Sciences, and were four weeks old at the beginning of our experiments. They were maintained in specific pathogen-free (SPF) facilities in our college and fed with irradiated chow. The two subclones of cells, K562-n and K562-n/VCR, were inoculated subcutaneously into each side of the back of athymic nude mice (5×10^6 cells/each) simultaneously, resulting in the formation of two tumors per mouse. The mice were assigned randomly to five groups

after inoculation for 10 days when both tumors formed. Mice in the control group were treated with normal saline 0.2 ml (group A). Nude mice in group B were treated with MNPs-Fe₃O₄ (0.58 mg/kg). Group C were treated with DNR (1 mg/kg). Group D were treated with MNPs-Fe₃O₄ (0.58 mg/kg) loaded with DNR (1 mg/kg) every other day for 20 days by vena caudalis injection, respectively.²⁸

Tumor assessment

After the planned treatment all animals were killed with ether anesthesia. Tumors were isolated for weight detection and then for quantitative real-time reverse transcription–polymerase chain reaction and western blotting.

Quantitative real-time reverse transcription–polymerase chain reaction

A total RNA of K562-n/VCR tumors from all groups and K562-n tumor of group A were isolated by using Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's protocol. One microgram of total RNA was used to generate cDNA by using SuperScript II Reverse Transcriptase (Invitrogen Life Technologies). PCR primers were designed to amplify products within target and control sequences (primer sequences for Mdr-1 (427 bp) forward, 5'-TGGT TGTATGTGCACGATGTTGGG-3' and reverse, 5'-AGATCAGCAGGAAAGCAGCACCTA-3'; Bcl-2 (452bp) forward, 5'-GGGAGAACAGGGTACGATAA-3' and reverse, 5'-CCACCGAACTCAAAGAAGG-3'; Bax (114bp) forward, 5'-TTTTGCTTCAGGGTTTCATC-3' and reverse, 5'-GACACTCGCTCAGCTTCTTG-3'; caspase-3 (445 bp) forward, 5'-CACAATAGCACCCATCCG-3' and reverse, 5'-GGGACATCAGTCGCTTCA-3'; GAPDH (205bp) forward, 5'-CGGATTTGGTCGTATTG-3' and reverse, 5'-GAAGATGGTGATGGGATT-3'). Quantitative PCR was performed by monitoring in real-time the increase in fluorescence of SYBR green I dye (Takara, Shiga, Japan) with Rotor-Gene 3000 (Corbett Research, Sydney, Australia). Each experiment was undertaken in triplicate. The relative gene copy number was calculated by the concentration-CT standard curve method and normalized using the average expression of GAPDH.

Western blot analysis

Western blotting was used for the detection of P-gp. After treatment for 20 days, 0.1 g K562-n/VCR tumor tissue was collected from each mouse and homogenized for P-gp detection, tumor tissue from K562n is used as negative control. Tissues were extracted with 1 g/L Triton X-100 and protein

concentration was determined with a Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA) and standardized with bovinserum albumin. A 50 µg sample of protein was separated on electrophorated SDS-PAGE and electroblotted onto PVDF membrane (BioRad Laboratories). The membrane was blocked with buffer containing 10% fat free dry milk. Mouse anti-Bcl-2 antibody (1:500), mouse anti-Bax antibody (1:500), mouse anti-Caspase-3 antibody (1:500), mouse anti-P-gp antibody (1:500) and mouse anti-β-actin antibody (1:500) were used respectively as the primary antibody. Horseradish peroxidase-conjugated anti-rabbit antibody (1:1000) was the secondary antibody. The band was detected by using an enhanced chemiluminescence (ECL) detection system.

Statistical analysis

For tumor diameter assessment and pathological examinations, experiments were repeated at least three times and evaluated by two independent researchers when indicated. Reported values represent the means ± SD. The significance of differences between experimental variables was determined using parametric Student's *t*-test.

Results

DNR-loaded MNPs-Fe₃O₄ could greatly suppress the growth of K562n/VCR tumor, but failed to further inhibit the proliferation of sensitive K562-n tumor *in vivo*.

All models of nude mice with transplanted leukemic K562-n cell and its MDR counterpart K562n/VCR were established after subcutaneous injection for 10 days, with an average volume of 100 mm³. The rate of subcutaneous tumor formation was 100%. For MDR-bearing K562-n/VCR tumors, there was no significant difference in the average tumor weight between groups A and B, the average tumor volume of group C seemed smaller but without statistic difference. The average tumor weight was less in group D than that in groups A, B, and C (*P* < 0.05) (Figure 1A). For sensitive K562-n tumors, there was no significant difference in the average tumor weight between groups A and B. The average tumor weight was less in groups C and D than that in groups A and B (*P* < 0.05). But the average tumor weight showed no difference in groups C and D (Figure 1B).

Transcription of Mdr-1, Bcl-2, Bax, and caspase-3 in K562-n/VCR tumor by RT-PCR

The transcription of Mdr-1 and Bcl-2 gene was significantly lower in group D than those in groups A, B, and C (group D vs groups A, B, or C; *P* < 0.05). The transcription of Bax and caspase-3

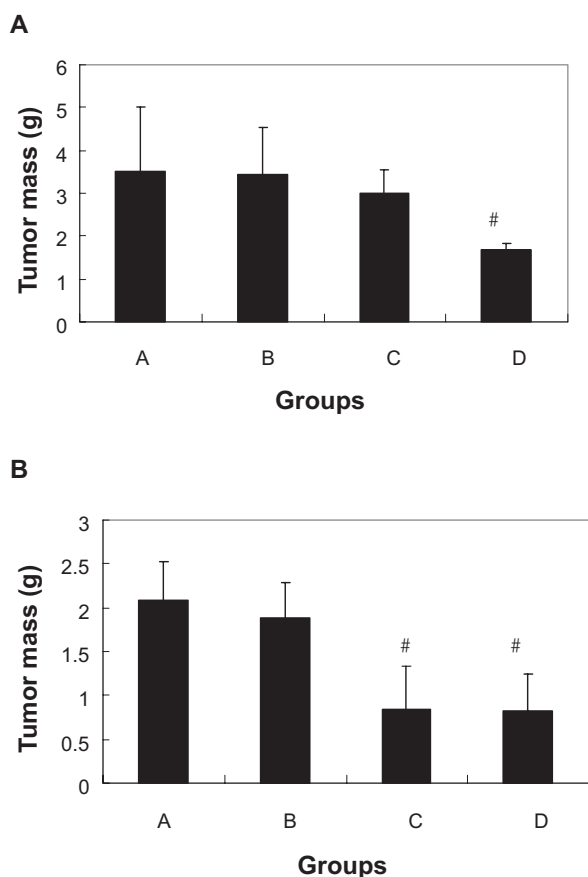


Figure 1 Tumor weight change in four groups. **A)** negative control, **B)** MNPs-Fe₃O₄, **C)** DNR; **D)** MNPs-Fe₃O₄ + DNR.

Notes: **A:** K562-n/VCR tumor mass; [#]*P* < 0.05, the average K562n/VCR tumor weight was less in group D than that in groups A, B and C; **B:** k562-n tumor mass. [#]*P* < 0.05, the average K562-n tumor weight was less in groups C and D than that in groups A and B.

Abbreviations: DNR, daunorubicin; MNPs-Fe₃O₄, magnetic nanoparticles of Fe₃O₄.

increased more significantly in group D than in groups A, B, and C (group D vs groups A, B, or C; *P* < 0.05) (Figure 2).

Expression of P-gp, Bcl-2, Bax, and caspase-3 proteins in K562-n/VCR tumor by western blot

Based on computer-assisted image analysis, it appeared that there was no difference in the P-gp expression among the four groups (Figure 3). The expression of Bcl-2 was significantly lower in group D than in groups A, B, and C (group D vs groups A, B, or C; *P* < 0.05). The expression of Bax and caspase-3 increased more significantly in group D than in groups A, B, and C (group D vs groups A, B, or C; *P* < 0.05) (Figure 4).

Discussion

In the present study, DNR-loaded MNPs-Fe₃O₄ could selectively inhibit the growth of MDR leukemic K562-n/VCR cells that transplanted in nude mice when compared

with DNR alone according to the average tumor weight (*P* < 0.05), while MNPs-Fe₃O₄ didn't show any antitumor activity without DNR.

Many chemotherapeutic agents target intracellular organelles or molecules to achieve their anticancer activities.²⁹ Effective chemotherapy thus requires an effectively high level of drug molecules to accumulate within the cancer cells. In addition, the effectiveness of chemotherapy is also correlated with drug exposure time.³⁰ In P-gp overexpressing cells, it becomes a difficult task to maintain a high intracellular drug level for a reasonable length of time. Experimental studies have shown that the main antitumor effect of anthracyclines is correlated to the induction of apoptotic cells.³¹ Escape from apoptotic signals often accompanies MDR and tumor progression.^{32,33} In general, apoptosis may occur through specific apoptosis signaling pathways such as death receptors and mitochondria. However, the mitochondrial pathway plays a crucial role in anthracycline-related apoptosis,³⁴ which is regulated by the Bcl-2 protein family.³⁵ Bcl-2 family proteins consist of antiapoptotic and pro-apoptotic members.³⁶ Previous reports have also documented that the ratio of antiapoptotic Bcl-2 to proapoptotic Bax protein determined, at least in part, the susceptibility of cells to a death signal,³⁷ and are used as a predictive marker for therapeutic response to radiotherapy.³⁸ It is well known that caspase-3 plays the central role in the initiation of apoptosis.³⁹ Our study showed that DNR-loaded MNPs-Fe₃O₄ could decrease the expression of Bcl-2 and increase the expression of Bax and caspase-3 in K562-n/VCR tumors (*P* < 0.05), indicating that this therapy can overturn poor response and induce apoptosis of MDR K562-n/VCR cells to anticancer drugs *in vivo*. Our outcomes clearly indicate that a MNPs-Fe₃O₄ drug delivery system can facilitate DNR accumulation in MDR cells and enhance apoptosis in MDR cells.

It has been reported that DNR can interact with DNA in the presence of Fe (III) ions which hampers the recognition of DNA by transcription factors and then inhibits transcription.⁴⁰ We also found that DNR-loaded MNPs-Fe₃O₄ could down-regulate the transcription of *mdr-1* gene. The downregulation of the transcription of *mdr-1* gene may attribute to the relatively high DNR concentration in K562-n/VCR, since MNPs-Fe₃O₄ alone is not able to inhibit the *mdr-1* gene transcription. On the contrary, there is no difference in the quantity of P-gp on K562-n/VCR tumors among the four groups. Therefore functionalized MNPs-Fe₃O₄ can enhance DNR accumulation inside MDR cells without interfering with the quantity of P-gp, a mechanism which differs from many types of P-gp modulators such as tetrandrine, PC833,

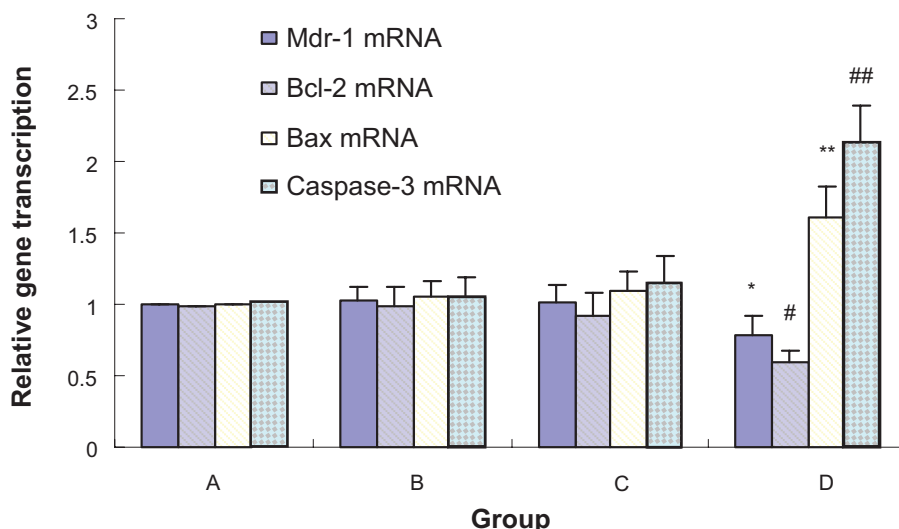


Figure 2 The transcription of Mdr-1, Bcl-2, Bax, and caspase-3 gene after treatment for 20 days detected by real time RT-PCR. **A)** negative control; **B)** MNPs-Fe₃O₄; **C)** DNR; **D)** MNPs-Fe₃O₄ + DNR.

Notes: * $P < 0.05$, the transcription of mdr-1 was significantly lower in group D than that in groups A, B, and C; # $P < 0.05$, the transcription of Bcl-2 gene was significantly lower in group D than that in group A, B and C; ** $P < 0.05$, the transcription of Bax was increased significantly in group D than that in group A, B and C; ## $P < 0.05$, the transcription of caspase-3 was increased significantly in group D than that in group A, B and C.

Abbreviations: DNR, daunorubicin; MNPs-Fe₃O₄, magnetic nanoparticles of Fe₃O₄.

and so on.⁴¹ Quantity or content, but not function, of P-gp can be detected by western blot. Studies have shown that some of the lipids and surfactants used in detergent-based formulations including Pluronic block copolymers and solid lipid nanoparticles possess intrinsic P-gp inhibitory activities.⁴² Looking at structural features, Pluronic block copolymers, solid lipid nanoparticles, and tetraheptylammonium-capped MNPs-Fe₃O₄ are similar in having hydrophobic segments. The literature suggests that nonionic surfactants may inhibit drug efflux transport through increased membrane fluidization, which induces changes in the conformation of P-gp and ATPase activity.⁴³ It is reasonable to infer from enhanced DNR accumulation inside the MDR cells that hydrophobic tetraheptyl segments capped outside MNPs-Fe₃O₄ can incorporate DNR in the lipid membrane and induce changes in membrane structure. The interrelationship between the membrane fluidization and the suppression of P-gp ATPase activity can be better understood in view of the current picture of P-gp structure describing P-gp that contains two transmembrane domains (TMDs) and two nucleotide ATP-binding domains (NBDs).² Structures of P-gp protein suggest that the two NBDs form a common binding site where the energy of ATP is harvested to promote efflux through a pore that is delineated by transmembrane helices.⁴⁴ Proper interaction of these two ATP-binding sites is crucial for the proper functioning of P-gp.⁴³ Therefore, the structural perturbations in the lipid membranes induced by MNPs-Fe₃O₄ may decrease the affinity of ATP to its binding site and interfere

with the ATPase activity. Not only have MNPs-Fe₃O₄ the ability to block P-gp function, but they also have the potential for aggregation and drug capsulation.^{42,45} Nanoparticles loaded with anticancer drugs could readily approach the cell membrane, leading to drug concentrations at the cell surface higher than those obtained with the same amount of free drug solution, which leads in turn to higher intracellular drug concentrations.^{46,47} It has been reported that it is more difficult for P-gp to remove the drug molecules from the cells when these molecules are associated with nanoparticles. DNR-loaded MNPs-Fe₃O₄ complexes are likely to be too large to be handled by P-gp and are consequently “trapped” within the cancer cells once they gain entrance into the targeted cells.⁴² This is another possible mechanism responsible for enhanced cellular drug retention. These mechanisms may be the reason that MNPs-Fe₃O₄, which has no cytotoxicity to cells, is able to reverse MDR. Another important observation of the present work is that DNR-loaded MNPs-Fe₃O₄ fail to further suppress the growth of K562-n tumor cells. The advantages of nanoparticles over solution appear to be augmented by the increases in P-gp levels of the treated cells. This phenomenon is consistent with recent studies of polymer-lipid hybrid nanoparticle systems and Pluronic block copolymers.^{45,48} However, these mechanisms are not able to explain the remarkable selectivity of DNR-loaded MNPs-Fe₃O₄ complexes with respect to MDR cells. Since MNPs-Fe₃O₄ may not completely inhibit the P-gp function, DNR-loaded MNPs-Fe₃O₄ complexes are able to enhance

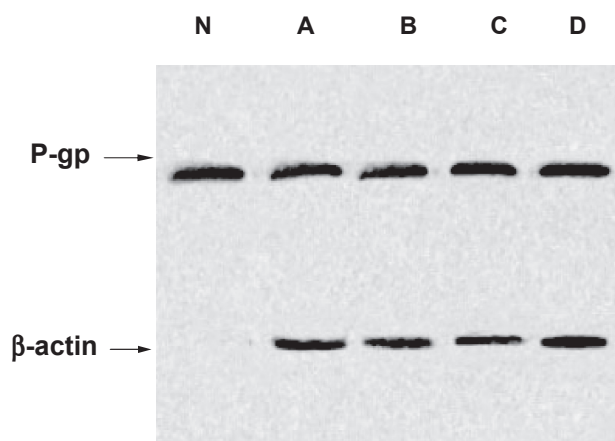


Figure 3 The expression of P-gp in K562n/VCR tumor after treatment for 20 days by western blot (K562n as a negative control).

Notes: $P > 0.05$, there was no difference in the expression of P-gp among those four groups. (A: negative control; B: MNPs- Fe_3O_4 ; C: DNR; D: MNPs- Fe_3O_4 + DNR).

the DNR accumulation in P-gp negative cells at the same time as the MDR counterpart. It has been demonstrated that DNR–nanoparticle conjugates are transported via a different mechanism than the free drug. The conjugate is most likely to be transported in the cells via endocytosis, which represents a P-gp-independent pathway, whereas the free drug is transported via membrane diffusion, a pathway affected by efflux pumps.⁴⁹ It also has been reported that endocytosis, secretion, and some other processes related to phase transitions in the plasma membrane are more active in MDR cells than in sensitive ones due to the higher membrane fluidity of the former.⁵⁰ This suggests that the increase in the drug uptake observed in MDR cells in the presence of MNPs- Fe_3O_4 was conditioned by the higher flexibility of

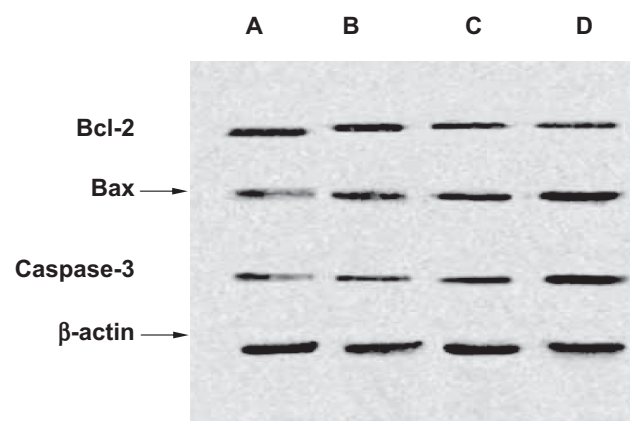


Figure 4 The expression of Bcl-2, Bax and caspase-3 proteins in K562-n/VCR tumor after treatment for 20 days by western blot.

Notes: $P < 0.05$, the expression of Bcl-2 was significantly lower in group D than that in group A, B and C; $P < 0.05$, the expression of Bax and Caspase-3 were increased significantly in group D than those in group A, B and C (A: negative control; B: MNPs- Fe_3O_4 ; C: DNR; D: MNPs- Fe_3O_4 + DNR).

their membranes. P-gp is normally expressed in the transport epithelium of the liver, kidney, and gastrointestinal tract, at pharmacological barrier sites, in adult stem cells, and in assorted cells of the immune system.^{51,52} A wide variety of substrates are translocated by P-gp transporters, ranging from chemotherapeutic drugs to naturally occurring biological compounds. It is becoming increasingly evident that P-gp has a pivotal role in host detoxification and protection of the body against xenobiotics. We wonder if the abrogation of P-gp by tetraheptylammonium-capped MNPs- Fe_3O_4 would result in systemic toxicity. It has been reported that *mdr-1a/1b* double knockout mice are viable and fertile and are almost indistinguishable from their wild-type littermates, which suggests that pharmacological modulation of human P-gp could represent a safe and effective strategy to thwart MDR cancers.⁵³ Our histological data show that the application of MNPs- Fe_3O_4 was well tolerated by nude mice.

In conclusion, our results support the proposition that MNPs- Fe_3O_4 offers an attractive means of delivering DNR into MDR tumor cells and enhances apoptosis in the MDR cells without reducing P-gp protein in the cell membrane. DNR-loaded MNPs- Fe_3O_4 complex is insensitive to P-gp-mediated drug efflux and is more effective than free DNR in MDR cells. In any case, the DNR-loaded MNPs- Fe_3O_4 complex may target the Achilles' heel of MDR cells.

Acknowledgments

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References

- Persidis A. Cancer multidrug resistance. *Nat Biotechnol.* 1999;17:94–95.
- Kohler N, Sun C, Wang J, et al. Methotrexate-modified superparamagnetic nanoparticles and their intracellular uptake into human cancer cells. *Langmuir.* 2005;21:8858–8864.
- Jozef H, Peter R, Jan H, et al. Genes of multidrug resistance in hematological malignancies. *Biologia.* 2006;5:219–234.
- Hendrik W, Abelardo M, Monique P, et al. Multidrug resistance in lactic acid bacteria: molecular mechanisms and clinical relevance. *Antonie Van Leeuwenhoek.* 1999;76:347–352.
- Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat Rev Cancer.* 2002;2:48–58.
- Ambudkar SV, Dey S, Hrycyna CA, Ramachandra M, Pastan I, Gottesman MM. Biochemical, cellular, and pharmacological aspects of the multidrug transporter. *Pharmacol Toxicol.* 1999;39:361–398.

7. Lehnert M. Multidrug resistance in human cancer. *J Neurooncol.* 1994;22:239–243.
8. Zhang RY, Wang XM, Wu CH, et al. Synergistic enhancement effect of magnetic nanoparticles on anticancer drug accumulation in cancer cells. *Nanotechnology.* 2006;17:3622–3626.
9. Martin W. Natural and synthetic polymers as inhibitors of drug efflux pumps. *Pharm Res.* 2008;25:500–511.
10. Sells RA, Owen RR, New RR, Gilmore IT. Reduction in toxicity of doxorubicin by liposomal entrapment. *Lancet.* 1987;2:624–625.
11. Rahman A, Treat J, Roh JK, et al. A phase I clinical trial and pharmacokinetic evaluation of liposome-encapsulated doxorubicin. *J Clin Oncol.* 1990;8:1093–1100.
12. Cowens JW, Creaven PJ, Greco WR, et al. Initial clinical (phase I) trial of TLC D-99 (doxorubicin encapsulated in liposomes). *Cancer Res.* 1993;53:2796–2802.
13. Gabizon A, Catane R, Uzieli B, et al. Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. *Cancer Res.* 1994;54:987–992.
14. Storm G, ten Kate MT, Working PK, Bakker-Woudenberg IA. Doxorubicin entrapped in sterically stabilized liposomes: effects on bacterial blood clearance capacity of the mononuclear phagocyte system. *Clin Cancer Res.* 1998;4:111–115.
15. Gref R, Minamitake Y, Peracchia MT, et al. Biodegradable long-circulating polymeric nanospheres. *Science.* 1994;263:1600–1603.
16. Couvreur P, Barratt G, Fattal E, et al. Nanocapsule technology: A review. *Crit Rev Ther Drug Carrier Syst.* 2002;19:99–134.
17. Muller RH, Keck CM. Challenges and solutions for the delivery of biotech drugs: a review of drug nanocrystal technology and lipid nanoparticles. *J Biotechnol.* 2004;113:151–170.
18. Ferrari M. Cancer nanotechnology: opportunities and challenges. *Nat Rev Cancer.* 2005;5:161–171.
19. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. *Nat Nanotechnol.* 2007;2:751–760.
20. Wang X, Zhang R, Wu C, et al. The application of Fe₃O₄ nanoparticles in cancer research: A new strategy to inhibit drug resistance. *J Biomed Mater Res A.* 2007;80:852–860.
21. Chen BA, Sun Q, Wang X, et al. Reversal in multidrug resistance by magnetic nanoparticle of Fe₃O₄ loaded with adriamycin and tetrandrine in K562/A02 leukemic cells. *Int J Nanomedicine.* 2008;3:277–286.
22. Cheng F, Su C, Yang Y, et al. Characterization of aqueous dispersions of Fe₃O₄ nanoparticles and their biomedical applications. *Biomaterials.* 2005;26:729–738.
23. Piseri P, Tafreshi HV, Milani P. Manipulation of nanoparticles in supersonic beams for the production of nanostructured materials. *Cur Opin Solid State Mater Sci.* 2004;8:195–202.
24. Gao H, Wang J, Shen X, et al. Preparation of magnetic polybutylcyanoacrylate nanospheres encapsulated with aclacinomycin A and its effect on gastric tumor. *World J Gastroenterol.* 2004;10:2010–2013.
25. Bennis S, Chapey C, Couvreur P, et al. Enhanced cytotoxicity of doxorubicin encapsulated in polyisohexylcyanoacrylate nanospheres against multidrug-resistant tumour cells in culture. *Eur J Cancer.* 1994;30:89–93.
26. Xu XP, Ding XJ, Yan Y, et al. Establishment and characterization of human leukemia cell lines having high tumorigenicity in nude mice. *Chin J Hematol.* 1996;17:142–145.
27. Chen L, Xu XP, Wang JM, et al. Establishment and characterization of human multidrug resistance leukemia cell lines having high tumorigenicity in nude mice. *Acad J Second Mil Med Univ.* 2004;25:507–511.
28. Gao L, Chen L, Fei XH, et al. STI571 combined with vincristine greatly suppressed the tumor formation of multidrug-resistant K562 cells in a human-nude mice xenograft model. *Chin Med J.* 2006;119: 911–918.
29. Dorr RT. Radioprotectants: Pharmacology and clinical applications of amifostine. *Semin Radiat Oncol.* 1998;8:10–13.
30. Millenbaugh NJ, Wientjes MG, Au JL. A Pharmacodynamic analysis method to determine the relative importance of drug concentration and treatment time on effect. *Cancer Chemother Pharmacol.* 2000;45:265–272.
31. Skladanowski A, Konopa J. Adriamycin and daunomycin induce programmed cell death (apoptosis) in tumour cells. *Biochem Pharmacol.* 1993;46:375–382.
32. Kerr JF, Winterford CM, Harmon BV. Apoptosis. Its significance in cancer and cancer therapy. *Cancer.* 1994;73:2013–2026.
33. Sartorius UA, Krammer PH. Upregulation of Bcl-2 is involved in the mediation of chemotherapy resistance in human small cell lung cancer cell lines. *Int J Cancer.* 2002;97:584–592.
34. Durrieu F, Belloc F, Lacoste P, et al. Caspase activation is an early event in anthracycline-induced apoptosis and allows detection of apoptotic cells before they are ingested by phagocytes. *Exper Cell Res.* 1998;240:165–175.
35. Adams JM, Cory S. The Bcl-2 protein family: arbiters of cell survival. *Science.* 1998;281:1322–1326.
36. Burlacu A. Regulation of apoptosis by Bcl-2 family proteins. *Cell Mol Med.* 2003;7:249–257.
37. Vander Heiden MG, Thompson CB. Bcl-2 proteins: regulators of apoptosis or of mitochondrial homeostasis. *Nat Cell Biol.* 1999;1: E209–E216.
38. Mackey TJ, Borkowski A, Amin P, et al. Bcl-2/bax ratio as a predictive marker for therapeutic response to radiotherapy in patients with prostate cancer. *Urology.* 1998;52:1085–1090.
39. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. *Science.* 1995;267:1456–1462.
40. Agata S, Marek G, Malgorzata C. Sequence specificity of formaldehyde-mediated covalent binding of anthracycline derivatives to DNA. *Biochem Pharmacol.* 2005;69:7–18.
41. Zhu X, Sui M, Fan W. *In vitro* and *in vivo* characterizations of tetrandrine on the reversal of P-glycoprotein-mediated drug resistance to paclitaxel. *Anticancer Res.* 2005;25:1953–1962.
42. Wong HL, Rauth AM, Bendayan R, et al. A new polymer-lipid hybrid nanoparticle system increases cytotoxicity of doxorubicin against multidrug-resistant human breast cancer cells. *Pharm Res.* 2006;23:1574–1585.
43. Batrakova EV, Li S, Vinogradov SV, et al. Mechanism of pluronic effect on P-Glycoprotein efflux system in blood-brain barrier: contributions of energy depletion and membrane fluidization. *J Pharmacol Exp Ther.* 2001;299:483–493.
44. Chang G, Roth CB. Structure of MsbA from *E. coli*: a homolog of the multidrug resistance ATP binding cassette (ABC) transporters. *Science.* 2001;293:1793–1800.
45. Wong HL, Bendayan R, Rauth AM, et al. A mechanistic study of enhanced doxorubicin uptake and retention in multidrug resistant breast cancer cells using a polymer-lipid hybrid nanoparticle system. *J Pharmacol Exp Ther.* 2006;317:1372–1381.
46. Soma CE, Dubernet C, Bentolila D, Benita S, Couvreur P. Reversion of multidrug resistance by co-encapsulation of doxorubicin and cyclosporin A in polyalkylcyanoacrylate nanoparticles. *Biomaterials.* 2000;21:1–7.
47. Hu Y, Jarillon S, Dubernet C, et al. On the mechanism of action of doxorubicin encapsulation in nanospheres for the reversal of multidrug resistance. *Cancer Chemother Pharmacol.* 1996;37:556–560.
48. Batrakova EV, Li S, Elmquist WF, et al. Mechanism of sensitization of MDR cancer cells by Pluronic block copolymers: Selective energy depletion. *Br J Cancer.* 2001;85:1987–1997.
49. Kopecek J, Kopecková P, Minko T, et al. HEMA copolymer anticancer drug conjugates: design, activity, and mechanism of action. *Eur J Pharm Biopharm.* 2000;50:61–81.
50. Arsenaut A, Ling V, Kartner N. Altered plasma membrane ultrastructure in multidrug resistant cells. *Biochim Biophys Acta.* 1988;938:315–321.
51. Cordon-Cardo C, O'Brien JP, Boccia J, Casals D, Bertino JR, Melamed MR. Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. *J Histochem Cytochem.* 1990;38:1277–1287.

52. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. Immunohistochemical localization in normal tissues of different epitopes in the multidrug transport protein P170: evidence for localization in brain capillaries and cross reactivity of one antibody with a muscle protein. *J Histochem Cytochem.* 1989;137:159–164.
53. Schinkel AH, Smit JJ, van Tellingen O, et al. Disruption of the mouse *mdr1a* P-glycoprotein gene leads to a deficiency in the blood–brain barrier and to increased sensitivity to drugs. *Cell.* 1994;77:491–502.

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