J Biosci (2019) 44:39
DOI: 10.1007/s12038-019-9854-x

© Indian Academy of Sciences

ABCG2 aptamer selectively delivers doxorubicin to drug-resistant breast cancer cells

SHIRIN HASHEMITABAR[1], REZVAN YAZDIAN-ROBATI[2], MARYAM HASHEMI[3], MOHAMMAD RAMEZANI[3,4], KHALIL ABNOUS[4,5]* and FATEMEH KALALINIA[1,6]*

[1]*Biotechnology Research Center, Institute of Pharmaceutical Technology, Mashhad University of Medical Sciences, Mashhad, Iran*

[2]*Molecular and Cell biology Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

[3]*Nanotechnology Research Center, Institute of Pharmaceutical Technology, Mashhad University of Medical Sciences, Mashhad, Iran*

[4]*Pharmaceutical Research Center, Institute of Pharmaceutical Technology, Mashhad University of Medical Sciences, Mashhad, Iran*

[5]*Medicinal Chemistry Department, Academy of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran*

[6]*Genetic Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*

**Corresponding author (Email, kalaliniaf@mums.ac.ir; abnouskh@mums.ac.ir)*

MS received 3 January 2018; accepted 17 January 2019; published online 9 April 2019

Chemotherapy is the most widely used treatment for cancer therapy, but its efficacy is limited by the side effects of non-specific cytotoxic drugs. Ligand-based targeting drug-delivery system is a solution to circumvent this issue. In this study, an ABCG2 aptamer–doxorubicin complex was prepared, and its efficacy in targeted drug delivery to mitoxantrone-resistance breast cancer cell line (MCF7/MX) was evaluated. The formation of aptamer–doxorubicin physical complex was analyzed by fluorometric analysis. The cytotoxicities of doxorubicin and aptamer–doxorubicin complex on MCF7 and MCF7/MX cell lines were evaluated by the MTT assay, and IC50 values were obtained. Cellular uptake of aptamer–doxorubicin complex was assessed by flow cytometry cellular uptake assay. Results: Fluorometric analysis of aptamer–doxorubicin showed 1–1.5 molar ratio of the drug to the aptamer could efficiently quench Dox fluorescence. MTT assay results showed that MCF7/MX cells were more resistant to doxorubicin than MCF7 cells ($IC_{50}: 3.172 \pm 0.536$ and 1.456 ± 0.154 μM, respectively). Flow cytometry and MTT assay results showed that the aptamer–doxorubicin complex could increase the uptake and cytotoxicity of doxorubicin in MCF7/MX cell line in comparison with free doxorubicin, while the same treatments had no effect on IC50 of Dox on MCF7 cells. The results proposed that the ABCG2 aptamer–drug complex can be effectively used for specific drug delivery to ABCG2-overexpressing cells.

Keywords. ABCG2; aptamer; breast cancer; doxorubicin; MDR

Abbreviations: ABCG2, ATP-Binding Cassette sub-family G member 2; APT, Aptamer; DMSO, Dimethyl sulfoxide; DOX, Doxorubicin; FBS, Fetal bovine serum; IC50, Inhibitory concentration 50%; MDR, Multi-drug resistance; MFI, Mean fluorescence intensity; MX, Mitoxantrone; PBS, Phosphate-buffered saline; SELEX, Systematic evolution of ligands by exponential enrichment

1. Introduction

Chemotherapy is one of the most widely used cancer therapies, but its efficacy is limited by different obstacles such as side effects of cytotoxic compounds on healthy tissues and the occurrence of drug resistance. One approach to circumvent these issues is to use targeted drug-delivery systems that selectively deliver drugs to cancer cells. Consequently, the cytotoxic effects of drugs against tumors are enhanced while their adverse effects on normal cells are reduced (Hu *et al.* 2012). A common targeted drug-delivery system involves an anticancer drug and a targeting ligand that specifically binds to tumor markers (Ye and Yang 2009). In this system, an ideal tumor marker should be a membrane protein that is abundantly expressed on the surface of cancer cells while it has no or low expression in normal tissues (Hu *et al.* 2012).

An ideal targeting ligand should bind to the tumor marker with reasonable specificity, high binding affinity and low immunogenicity (Vasir and Labhasetwar 2005).

Undoubtedly, breast cancer is the most frequently occurring cancer among women across the globe. Many researchers are trying to develop more sensitive methods to deal with this cancer (Ferlay *et al.* 2010). For many decades, doxorubicin has been widely utilized in the treatment of breast cancer in the early or metastatic stage, but it showed intrinsic cardiotoxicity that limited its usage (Lao *et al.* 2013). A major problem limiting the efficiency of chemotherapy is Multi-drug resistance phenomenon that is caused by different complex mechanisms. Multi-drug resistance phenomenon that is caused by different complex mechanisms is the main factor that limits the efficiency of chemotherapy agents (Sonneveld *et al.* 1992). Overexpression of adenosine triphosphate-binding cassette (ABC) transporters such as ABCG2 has been known as the most probable reason for drug resistance (Hirschmann-Jax *et al.* 2005; Song and Miele 2007). ABCG2 or breast cancer resistance protein (BCRP) is an ABC efflux transporter (Mao and Unadkat 2015) that extrudes a wide variety of therapeutic agents and physiological substances from the cells. Now, ABCG2 has been known as one of the vital drug transporters that is involved in clinical drug resistance phenotype (Staud and Pavek 2005; Mao and Unadkat 2015).

On the contrary, it has been shown that ABC transporters, including ABCG2 are highly expressed in cancer stem cells and make them resistant to chemotherapy agents (Staud and Pavek 2005; Mao and Unadkat 2015). Consequently, cancer stem cells can remain alive after chemotherapy, differentiate into mature tumor cells and finally develop a multidrug-resistant cancer (Stacy *et al.* 2013). Therefore, it seems those drug-delivery systems that selectively target the ABCG2 overexpressing cells, will be a successful chemotherapy procedure (Jonker *et al.* 2005; Staud and Pavek 2005; Hu *et al.* 2012).

Aptamers are short single-stranded (ss) oligonucleotides with unique intramolecular conformations. They are isolated through *in vitro* selection process termed systematic evolution of ligands by exponential enrichment (SELEX) against a wide variety of targets, including small molecules, ions, proteins, antibiotics or even whole cells (Zhu *et al*. 2012; Robati *et al.* 2016). In 2012, Palaniyandi *et al.* successfully developed the human ABCG2-specific aptamer (Palaniyandi *et al.* 2012). This study aimed to prepare a targeted drug-delivery system using the ABCG2 aptamer that specifically delivered doxorubicin to the breast cancer ABCG2-overexpressing cell line MCF7/MX (Kalalinia *et al.* 2014).

2. Materials and methods

2.1 *Materials*

ABCG2 aptamer (5′-GCTCGGATGCCACTACAGGCCCACCCTCATGGACGTGCTGGTGAC-3′) was purchased from Bioneer (South Korea). 2,5-Diphenyltetrazolium bromide (MTT) was obtained from Sigma-Aldrich (Germany), and doxorubicin (DOX) was obtained from Pfizer (USA). RPMI 1640 was bought from Biosera (France), fetal bovine serum (FBS) and penicillin-streptomycin were obtained from Sigma (USA).

2.2 *Cell lines and cell culture*

The previously isolated ABCG2-overexpressing MCF 7/MX cell line (Nakagawa *et al.* 1992), and its parental line, MCF-7 were generously provided by Dr Erasmus Schneider (Wadsworth Center, New York State Department of Health, USA) (Kalalinia *et al.* 2014). Cells were cultured in RPMI 1640 supplemented with FBS 10% (v/v), penicillin (100 units/mL), and streptomycin (100 µg/mL) at 37°C in a humidified incubator containing 5% CO_2. To maintain the multidrug-resistant phenotype, MCF-7/MX cells were cultured in the presence of mitoxantrone (MX) 100 nM that change to MX-free medium at least seven days before each experiment (Nakagawa *et al.* 1992; Kalalinia *et al.* 2014).

2.3 *Doxorubicin loading onto the ABCG2 aptamer*

To evaluate doxorubicin (DOX) loading onto the ABCG2 aptamer, increasing concentrations of aptamer (0.05–4 µM) were added to a constant concentration of DOX (1 µM) in phosphate-buffered saline (PBS) for 1 h at room temperature. The fluorescence spectra were measured by Synergy H4 microplate reader (BioTek, USA) (λ_{Ex} = 480 nm, λ_{Em} = 530–700 nm) (*Yazdian-Robati et al*. 2016).

2.4 *MTT assay*

The cell viability in the presence of DOX and Apt-DOX conjugate was evaluated by MTT assay. MCF7 and MCF7/MX cells (10^4 cells per well) were seeded in 96-well plate. After 24 h, sub-confluent cells were changed to fresh FBS-free culture medium containing DOX (0–3 µM) and Apt-DOX conjugate (with the same concentration of DOX). Cells were incubated at 37°C for 48 h. Then, the medium was removed and replaced by 100 µL of 0.5 mg/mL of MTT and the plates were incubated at 37°C for 4 h. Finally, supernatants were removed, and 100 µL DMSO was added to each well to solubilize the reduced MTT dye, and absorbance was measured using the microplate reader (BioTek, USA) at 570 and 630 nm. The percentage of viable cells was calculated by the ratio of $OD_{test}/OD_{control} \times 100$ (Kalalinia *et al.* 2011).

2.5 *Flow cytometric analysis of doxorubicin accumulation*

To study the drug accumulation, sub-confluent MCF7 or MCF7/MX cells were washed with PBS, trypsinized, and 25×10^4 of these cells were transferred to tubes and centrifuged. The cell pellets were re-suspended in 1 mL of serum-free medium (RPMI-1640) containing DOX 1 μM or Apt-DOX conjugate (1 μm DOX-equivalent). Then, they were incubated at 37°C for 3 h. After washing with PBS, the cells were fixed by formaldehyde 4% for 10 min. Then the cells were rewashed and re-suspended in 0.5 mL ice-cold PBS and kept on ice until flow cytometric analysis. Accumulation of doxorubicin was measured using FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA) and analyzed by the Cell Quest software (Becton Dickinson). Data from 1×10^4 cells were collected and analyzed with FlowJo 7.6.1 software (Mosaffa *et al.* 2012; Zeng *et al.* 2014).

2.6 *Statistical analysis*

Statistical analyses were performed by the 'Graph Pad Prism 6' using ANOVA for multiple group comparisons with Dunnett's post hoc test. Results are expressed as means ± SD of at least three independent experiments. *P* values less than 0.05 were considered statistically significant.

3. Results

3.1 *Characterization of Apt-DOX conjugate*

Apt-DOX conjugate formation was assessed by fluorometric analysis. DOX is an intercalating agent, and its fluorescence feature is quenched upon its intercalation to DNA (Mohan and Rapoport 2010). Figure 1 shows the quenching profile of DOX fluorescence spectra by adding increasing concentrations of Apt (0.05–4 μM) to a constant concentration of DOX (1 μM). Results indicated that the maximum quenching of DOX occurred at approximately 1:1.5-mole ratio of DOX to Apt.

3.2 *Cytotoxicity of doxorubicin on MCF7 and MCF7/MX cell lines*

MTT assay was performed to measure the cytotoxicity of DOX on the MCF7 and MCF7/MX cell lines. The cells were incubated in the presence or absence of various concentrations of DOX (0–5 μM) for 48 h. DOX showed inhibitory effects on the cell viability rate of both MCF7 and MCF7/MX cell lines in a concentration-dependent manner (figure 2a and b). DOX showed more cytotoxic effect on parental cell line MCF7 (IC_{50} = 1.4 μM) in comparison with drug resistance cell line MCF7/MX (IC_{50} = 3.2 μM).

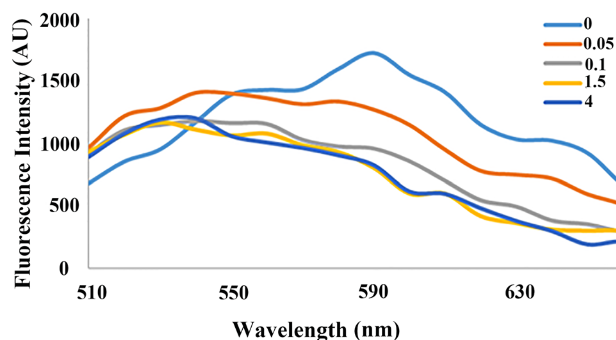

Figure 1. Fluorescence spectra of DOX upon interaction with ABCG2-aptamer (from top to bottom 0, 0.05, 0.1, 1.5 and 4 μM). The results indicated that the maximum quenching of DOX occurred at approximately 1:1.5 mol ratio of DOX to Apt.

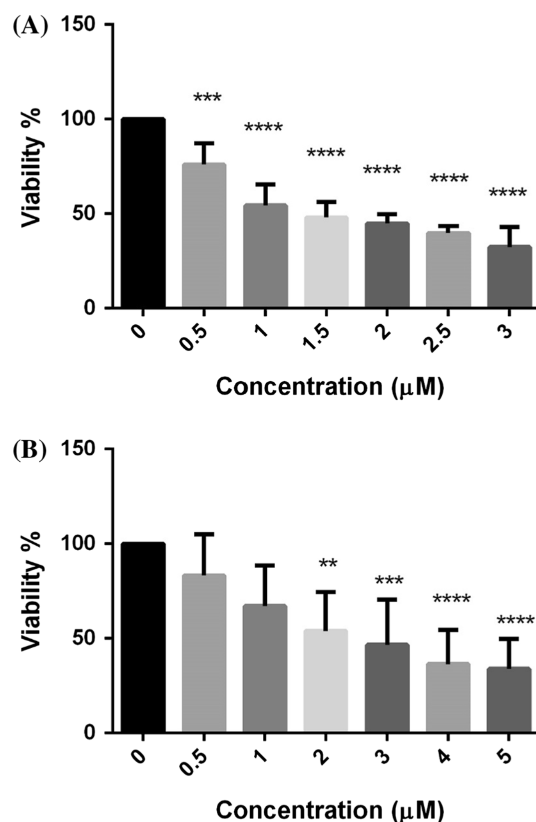

Figure 2. Effects of DOX on the viability rate of (**A**) MCF-7 and (**B**) MCF7/MX. In 96-well plates, 10^4 cells/well were plated in triplicate for overnight and then treated with DOX. After 48 h, the cell viability was determined by the MTT method and expressed as relative cell viability rate to control. The values are shown as mean ± SD. ***P* value ≤0.01, ****P* value ≤0.001 and *****P* value ≤0.0001.

3.3 *Selective delivery of doxorubicin to ABCG2 over-expressing cells*

The selective delivery of DOX to ABCG2-overexpressing MCF7/MX cell line was evaluated by the uptake study. For this purpose, the fluorescence generated by doxorubicin after

Table 1. Mean fluorescence intensity (MFI) of DOX in uptake study

	MFI (mean ± SD)	
	MCF7	MCF7/MX
Control	2760 ± 1.41	1488 ± 26.16
Doxorubicin	6377 ± 139.3	9560 ± 1018.2
Apt-Doxorubicin	6136 ± 84.5	13,000 ± 424.2**

**P value ≤0.01.

incubating the parental and resistant cell lines with free DOX or Apt-DOX was measured by flow cytometry. The results showed that the fluorescent signals generated by free DOX or Apt-DOX were similar in MCF7 cells (table 1 and figure 3A). Whereas, in ABCG2 over-expressing cell line MCF7/MX the fluorescent signal generated by Apt-DOX was significantly higher than that generated by free DOX (table 1 and figure 3B).

3.4 *Cytotoxicity of Apt-DOX complex on MCF7 and MCF7/MX cell*

Since the uptake of DOX was enhanced in the ABCG2 over-expressing cells, it was expected that its cytotoxicity to these cells would also be increased. To evaluate this hypothesis, the cytotoxicity caused by ABCG2 Apt-DOX or free DOX was compared in MCF7 and MCF7/MX cell lines. The results showed that treatment of MCF7/MX and MCF7 cells with aptamer did not have any significant effect on their cell viability. The viability rate of MCF7/MX cells under treatment with Apt-DOX conjugate (1 μm DOX-equivalent) was significantly lower than those treated by free DOX 1 μM (29.35 and 67.54%, respectively) (figure 4A). However, for parental non-resistance cell line MCF7, no significant difference was detected between the cytotoxicity caused by free DOX or Apt-DOX (the rate was 56.01 and 53.89%, respectively) (figure 4B).

4. Discussion

Chemotherapy has been known as the main strategy for cancer treatment, especially in metastatic cancers. However, its efficacy is often inadequate as a result of limited accessibility to the tumor tissue, development of multi-drug resistance, and harmful adverse effects due to their cytotoxic effects on the normal cells (Vasir and Labhasetwar 2005; Hu *et al.* 2012). One strategy for overcoming these issues is using targeted drug-delivery systems, consisting of a drug, a ligand component and a transport vehicle (Ye and Yang 2009) that individually distribute the sufficient concentration of drugs to the target cell or organ (Ye and Yang 2009).

ABCG2 is a member of ATP-binding cassette (ABC) efflux transporters that have been known as one of the critical transporter involved in drug resistance phenomenon. Therefore, it seems that ABCG2 could be the available target for ligand-guided anticancer drug delivery due to its over-expression in drug resistance tumor and cancer stem cells (Mao and Unadkat 2015). Aptamers are novel targeting ligands that preferred to other targeting agents based on their unique properties like limited synthesis cost, low-immunogenicity, high affinity to target molecules and small size that lets them penetrate the solid tumors (Osborne *et al.* 1997). Doxorubicin (DOX) rank among the most effective anti-cancer drugs for breast cancer, soft-tissue sarcomas, solid childhood tumors and aggressive lymphomas. However, the clinical use of DOX is limited by some serious problems such as the development of multidrug resistance in tumor cells and toxicity in healthy tissues especially cardiotoxicity (Minotti *et al.* 2004). Here in this study, we aimed to deliver the DOX to breast cancer resistance cell line specially. Therefore, we developed an ABCG2 aptamer–doxorubicin complex (ABCG2 Apt-DOX) by intercalating DOX into the DNA structure of the ABCG2 aptamer. A previously isolated mitoxantrone-resistant human MCF-7 breast cancer sub-line (MCF/MX) was used as ABCG2 over expressing cell line. It has already been shown that ABCG2 is over expressed up to 6000-fold in MCF7/MX cells when compared with MCF7 parental cell line, that was also confirmed in our previous

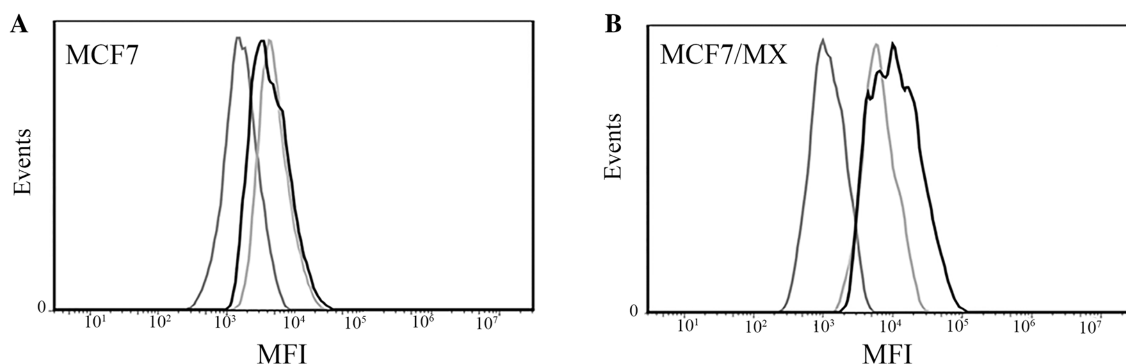

Figure 3. Uptake of doxorubicin by (**A**) ABCG2 over-expressing cell line (MCF7/mx) and (**B**) parental cell line (MCF7). Flow cytometry histogram profiles of DOX fluorescence intensity was obtained after incubation with either free DOX (light gray curves) or ABCG2 Apt-DOX (black curves). The unstained control sample (dark gray cures) has been used to detect auto fluorescence.

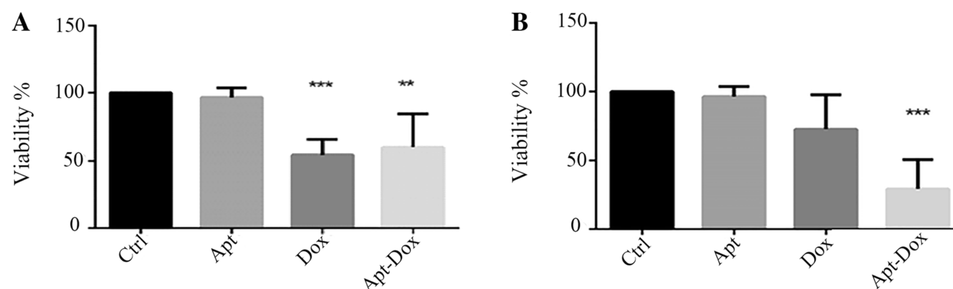

Figure 4. Cytotoxicity of Apt-DOX complex on MCF7 and MCF7/MX cell. In 96-well plates, 10^4 cells/well were plated in triplicate for overnight and then treated with free DOX (1 μM), APT (1.5 μM) or APT-DOX (1.5 μM/1 μM) for 48 h. After that, the cell viability was determined by the MTT method. The values are shown as mean ± SD. $^{**}P$ value $\leq$0.01 and $^{***}P$ value $\leq$0.001.

study (about 800-fold increase) (Kowalski *et al.* 2004; Kalalinia *et al.* 2014). Also, they indicated that MCF/MX is approximately 4000-fold resistant to mitoxantrone and also shows nearly 10-fold cross-resistant to doxorubicin (Nakagawa *et al.* 1992).

Previously, it has been proposed that covalent conjugation of DOX to the aptamer may decrease the DOX efficacy by reducing its release from aptamer and altering its structure (Senter *et al.* 1995). Therefore, in this study, the DOX was physically intercalated into the DNA structure of the ABCG2 aptamer, and this interaction was assessed by recording the quenching profile of DOX fluorescence. Previously, Haj *et al.* described a phenomenon that the fluorescence from DOX would be reduced after intercalating into DNA (Haj *et al.* 2003). About 64% decrease in DOX fluorescence intensity confirmed the formation of Apt-DOX conjugate and the maximum DOX quenching occurred at 1–1.5 molar ratio of drug to aptamer (figure 1).

Evaluation of the cytotoxic effects of DOX on the MCF7 and MCF7/MX cell lines showed that DOX inhibited the cell viability rate of both cell lines with higher intensity on parental drug-sensitive cell line MCF7 (IC_{50} = 1.4 μM) in comparison with drug-resistant cell line MCF7/MX (IC_{50} = 3.2 μM). It has been previously shown that ABCG2 expression level in resistance cell line MCF7/MX is notably higher than its expression level in non-resistance parental cell line MCF7 (Ross *et al.* 1999; Lao *et al.* 2013; Kalalinia *et al.* 2014). On the other hand, different studies showed that DOX is a substrate of ABCG2 transporter (Natarajan *et al.* 2012; Mao and Unadkat 2015). Therefore, it could be concluded that ABCG2 efflux DOX from the MCF7/MX cells that resulted in lower intercellular concentration and toxicity of DOX in this cell line in comparison with the parental non-resistance cell line.

The primary reason for adverse effects of DOX against normal tissue is non-selective uptake of free DOX by both normal and cancer cells. Lately, different studies tried to selectively deliver DOX to breast cancer drug resistance cells by specific aptamers. Jeong *et al.* developed a doxorubicin-incorporated mucin-1 aptamer-BCl2-specific siRNA conjugates and successfully transfected it to mucin-1 over expressing breast cancer multidrug-resistant cells. They showed that Apt-DOX complex exerts promising anticancer effects on multidrug-resistant cancer cells because of their higher intracellular uptake efficiency (Jeong *et al.* 2016). Liu *et al.* and Liao *et al.* designed and developed a nanostructure composed of DNA aptamer that targets cancer cells by binding with nucleolin. They could efficiently intercalate DOX into dsDNA with excellent stability. Apt-DOX nanoparticles effectively increased cell uptake and decreased cell efflux of doxorubicin that finally caused the inhibition of the resistance of human breast cancer cells to DOX. Interestingly, Apt-DOX nanoparticles could effectively inhibit tumor growth by less cardiotoxicity (Liao *et al.* 2015; Liu *et al.* 2016).

In this study, it was proposed that DOX are intercalating into the DNA structure of the ABCG2 aptamer (Apt-DOX), causes it preferentially to bind to ABCG2 over expressing cancer cells. The selective delivery of DOX was evaluated by uptake study and MTT assay. In the uptake study, flow cytometry analyses indicated that treatment of MCF7/MX cells with ABCG2 Apt-DOX complex significantly enhanced the fluorescence intensity of intercellular DOX in MCF7/MX cells in comparison with treatment with free DOX, while there were no significant differences in MCF7 cells. On the other hand, it was mentioned that the fluorescence from DOX was reduced about 64% after intercalating into aptamer, so the actual concentration of DOX in Apt-DOX treated MCF7/MX would be more than two-fold of that seen in DOX-treated MCF7/MX. Similarly, though the mean fluorescence intensity was similar in MCF7 cells treated with DOX and those treated with Apt-DOX, the actual concentration of DOX in MCF7 treated with Apt-DOX would be more but not significant.

The results of MTT assays confirmed those of the uptake study, where the cell viability of MCF7/MX treated with Apt-DOX significantly decreased in comparison with those treated with free DOX. While no significant difference was detected between the cytotoxicity caused by free DOX or Apt-DOX for parental non-resistance cell line MCF7, considering the previous prediction, it has been shown that higher expression of ABCG2 in MCF7/MX compared with the parental cell line, resulting in a lower concentration of DOX and subsequently higher viability of DOX-treated

MCF7/MX than DOX-treated MCF7 (67.54 and 53.89%, respectively). While, the cytotoxicity caused by Apt-DOX in MCF7/MX was much higher than MCF7 cells (with a viability rate 29.35 and 56.01%, respectively). These results proposed that ABCG2 Apt-DOX could distinguish between target and non-target cells.

In summary, in this study, ABCG2 aptamer-DOX was used to selectively deliver the cytotoxic agent doxorubicin to ABCG2 over expressing breast cancer resistance cell line MCF7/MX. Since ABCG2 is overexpressed on the surface of many tumor cells especially on the surface of the cancer stem cells, the aptamer could be a preferable guiding ligand for targeted chemotherapy against drug resistance malignancies.

Acknowledgements

The research presented in this paper was part of a Pharm.D thesis of Shirin Hashemitabar. The authors are indebted to the Research Council of Mashhad University of Medical Sciences, Iran, for approval and financial support of this project (Grant No. 940164).

References

Ferlay J, Shin HR, Bray F, Forman D, Mathers C and Parkin DM 2010 Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int. J. Cancer* **127** 2893–2917

Haj HT, Salerno M, Priebe W, Kozlowski H and Garnier-Suillerot A 2003 New findings in the study on the intercalation of bisdaunorubicin and its monomeric analogs with naked and nucleus DNA. *Chem. Biol. Interact.* **145** 349–358

Hirschmann-Jax C, Foster A, Wulf G, Goodell M and Brenner M 2005 A distinct 'side population' of cells in human tumor cells: implications for tumor biology and therapy. *Cell Cycle* **4** 206–208

Hu Y, Duan J, Zhan Q, Wang F, Lu X and Yang X-D 2012 Novel MUC1 aptamer selectively delivers a cytotoxic agent to cancer cells in vitro. *PLoS ONE* **7** e31970

Jeong H, Lee SH, Hwang Y, Yoo H, Jung H, Kim SH and Mok H 2017 Multivalent aptamer-RNA conjugates for simple and efficient delivery of doxorubicin/siRNA into multidrug-resistant cells. *Macromol. Biosci.* **17**(4) 1600343

Jonker JW, Merino G, Musters S, Van Herwaarden AE, Bolscher E, Wagenaar E, Mesman E, Dale TC and Schinkel AH 2005 The breast cancer resistance protein BCRP (ABCG2) concentrates drugs and carcinogenic xenotoxins into milk. *Nat. Med.* **11** 127–129

Kalalinia F, Elahian F and Behravan J 2011 The potential role of cyclooxygenase-2 on the regulation of the drug efflux transporter ABCG2 in breast cancer cell lines. *J. Cancer Res. Clin. Oncol.* **137** 321–330

Kalalinia F, Elahian F, Mosaffa F and Behravan J 2014 Celecoxib Up-regulates the expression of drug efflux transporter ABCG2 in breast cancer cell lines. *Iran. J. Pharm. Res.* **13** 1393–1401

Kowalski P, Farley KM, Lage H and Schneider E 2004 Effective knock down of very high ABCG2 expression by a hammerhead ribozyme. *Anticancer Res.* **24** 2231–2235

Lao J, Madani J, Puertolas T, Alvarez M, Hernandez A, Pazo-Cid R, Artal A and Anton Torres A 2013 Liposomal doxorubicin in the treatment of breast cancer patients: a review. *J. Drug Deliv.* **2013** 456409

Liao ZX, Chuang EY, Lin CC, Ho YC, Lin KJ, Cheng PY, Chen KJ, Wei HJ and Sung HW 2015 An AS1411 aptamer-conjugated liposomal system containing a bubble-generating agent for tumor-specific chemotherapy that overcomes multidrug resistance. *J. Control. Release* **208** 42–51

Liu J, Wei T, Zhao J, Huang Y, Deng H, Kumar A, Wang C, Liang Z, Ma X and Liang XJ 2016 Multifunctional aptamer-based nanoparticles for targeted drug delivery to circumvent cancer resistance. *Biomaterials* **91** 44–56

Mao Q and Unadkat JD 2015 Role of the breast cancer resistance protein (BCRP/ABCG2) in drug transport—an update. *AAPS J.* **17** 65–82

Minotti G, Menna P, Salvatorelli E, Cairo G and Gianni L 2004 Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacol. Rev.* **56** 185–229

Mohan P and Rapoport N 2010 Doxorubicin as a molecular nanotheranostic agent: effect of doxorubicin encapsulation in micelles or nanoemulsions on the ultrasound-mediated intracellular delivery and nuclear trafficking. *Mol. Pharm.* **7** 1959–1973

Mosaffa F, Kalalinia F, Lage H, Afshari JT and Behravan J 2012 Pro-inflammatory cytokines interleukin-1 beta, interleukin 6, and tumor necrosis factor-alpha alter the expression and function of ABCG2 in the cervix and gastric cancer cells. *Mol. Cell. Biochem.* **363** 385–393

Nakagawa M, Schneider E, Dixon KH, Horton J, Kelley K, Morrow C and Cowan KH 1992 Reduced intracellular drug accumulation in the absence of P-glycoprotein (mdr1) overexpression in mitoxantrone-resistant human MCF-7 breast cancer cells. *Cancer Res.* **52** 6175–6181

Natarajan K, Xie Y, Baer MR and Ross DD 2012 Role of breast cancer resistance protein (BCRP/ABCG2) in cancer drug resistance. *Biochem. Pharmacol.* **83** 1084–1103

Osborne SE, Matsumura I and Ellington AD 1997 Aptamers as therapeutic and diagnostic reagents: problems and prospects. *Curr. Opin. Chem. Biol.* **1** 5–9

Palaniyandi K, Pockaj BA, Gendler SJ and Chang X-B 2012 Human breast cancer stem cells have significantly higher rate of clathrin-independent and caveolin-independent endocytosis than the differentiated breast cancer cells. *J. Cancer Sci. Ther.* **4** 214

Robati RY, Arab A, Ramezani M, Langroodi FA, Abnous K and Taghdisi SM 2016 Aptasensors for quantitative detection of kanamycin. *Biosens. Bioelectron.* **82** 162–172

Ross DD, Yang W, Abruzzo LV, Dalton WS, Schneider E, Lage H, Dietel M, Greenberger L, Cole SPC and Doyle LA 1999 Atypical multidrug resistance: Breast cancer resistance protein messenger RNA expression in mitoxantrone-selected cell lines. *J. Natl. Cancer Inst.* **91** 429–433

Senter PD, Svensson HP, Schreiber GJ, Rodriguez JL and Vrudhula VM 1995 Poly(ethylene glycol)-doxorubicin conjugates containing beta-lactamase-sensitive linkers. *Bioconjug. Chem.* **6** 389–394

Song LL and Miele L 2007 Cancer stem cells—an old idea that's new again: implications for the diagnosis and treatment of breast cancer. *Expert Opin. Biol. Ther.* **7** 431–438

Sonneveld P, Lowenberg B, Durie B, Lokhorst H, Marie J, Zittoun R, Solbu G, Suciu S and Nooter K 1992 Modulation of multidrug-resistant multiple myeloma by cyclosporin. *Lancet* **340** 255–259

Stacy AE, Jansson PJ and Richardson DR 2013 Molecular pharmacology of ABCG2 and its role in chemoresistance. *Mol. Pharmacol.* **84** 655–669

Staud F and Pavek P 2005 Breast cancer resistance protein (BCRP/ABCG2). *Int. J. Biochem. Cell Biol.* **37** 720–725

Vasir JK and Labhasetwar V 2005 Targeted drug delivery in cancer therapy. *Technol. Cancer Res. Treat.* **4** 363–374

Yazdian-Robati R, Ramezani M, Jalalian SH, Abnous K and Taghdisi SM 2016 Targeted delivery of epirubicin to cancer cells by polyvalent aptamer system in vitro and in vivo. *Pharm. Res.* **33**(9) 2289–2297

Ye X and Yang D 2009 Recent advances in biological strategies for targeted drug delivery. *Cardiovasc. Hematol. Disord. Drug Targets* **9** 206–221

Zeng X, Morgenstern R and Nystrom AM 2014 Nanoparticle-directed sub-cellular localization of doxorubicin and the sensitization breast cancer cells by circumventing GST-mediated drug resistance. *Biomaterials* **35** 1227–1239

Zhu G, Ye M, Donovan MJ, Song E, Zhao Z and Tan W 2012 Nucleic acid aptamers: an emerging frontier in cancer therapy. *Chem. Commun.* **48** 10472–10480

Corresponding editor: RITA MULHERKAR