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Cationic submicron emulsions overcome multidrug resistance in SGC7901/VCR cells

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The over-expression of P-glycoprotein (P-gp) is associated with the development of multi-drug resistance (MDR) in cancer cells. In this study, we examined whether cationic submicron emulsions (CSEs) can efficiently deliver hydroxycamptothecin (HCPT) into MDR cells (SGC7901/VCR cells) via electrostatic-mediated endocytosis, thus overcoming MDR. We prepared HCPT-CSEs and rhodamine-123-CSEs (RH-123-CSEs), and examined the *in vitro* cytotoxic activity of HCPT-CSEs and the intracellular accumulation of HCPT and RH-123 in SGC7901/VCR cells. The HCPT-CSEs significantly increased the intracellular accumulation of HCPT (8.2-fold higher than HCPT-injection) and enhanced cytotoxic activity of HCPT (2.7-fold higher than HCPT-injection with verapamil). The fluorescence microscopic and flow cytometric detection on RH-123 supported the intracellular accumulation effect of CSEs. These results indicate CSEs may enhance drug-CSEs internalization followed by releasing their contents into the cytoplasm (near nuclear), thus lowering P-gp-mediated drug efflux. Furthermore, these *in vitro* results suggest that CSEs are a potentially useful drug delivery system to circumvent P-gp-mediated MDR of tumor cells.

1. Introduction

Resistance of tumor cells to chemotherapeutic agents is factually bothering in tumor therapy. Multi-drug resistance (MDR) is the most important form of drug resistance characterized by decreased cellular sensitivity and lowered drug intracellular concentration to a broad range of chemotherapeutic drugs (Breuninger et al. 1995; Lucci et al. 1999; Gottesman et al. 2002).

Tumor cells are developing drug resistance by over expression of drug efflux transporters like P-glycoprotein (P-gp) (Krishnamachary and Center 1993), changes in topoisomerase activity (Deffie et al. 1989), modifications in glutathione *S*-transferase (Zhang et al. 1998), and altered expression of apoptosis-associated protein Bcl-2 (Kirkin et al. 2004) and tumor suppressor protein p53 (Viktorsson et al. 2005). Among these, classic MDR is mainly caused by over-expression of P-gp, which is coded by the MDR1 gene and functions as an ATP-dependent drug-efflux membrane transporter that rapidly extrudes a variety of hydrophobic anticancer drugs from exerting their cytotoxic effects, and leads to reduced intracellular drug accumulation and decreased therapeutic efficacy (Krishnamachary and Center 1993; Nobili et al. 2006). Lots of drugs are affected by classic MDR including the Vinca alkaloids (vinblastine and vincristine), anthracyclines (doxorubicin and daunorubicin), the RNA transcription inhibitor actinomycin D, and the microtubule-stabilizing drug paclitaxel (Gottesman et al. 2002).

Colloidal carriers with anti-tumor drugs encapsulated in have been observed capable for overcoming multi-drug resistance in tumor cells, such as polymer nanoparticles (Soma et al. 2000; Vauthier et al. 2003; Barraud et al. 2005; Chavanpatil et al. 2006; Garcion et al. 2006), liposomes (Seid et al. 1991; Thierry et al. 1993; Krishna and Mayer 1999; Mayer and Shabbits 2001; Shabbits et al. 2001; Gatouillat et al. 2007), and block copolymer micelles (Batrakova et al. 1999; Lee et al. 2005; Dabholkar et al. 2006; Na 2007). Submicron emulsion (SE) is also colloidal drug delivery system and a potent candidate in sustained-release and targeted delivery systems. It has been previously shown that Cationic submicron emulsions (CSEs) are stable in the presence of physiological cations and can interact *in vivo* with negatively charged biological membranes, resulting in an enhanced drug uptake as compared to the anionic emulsion (Shi and Benita 2000; Goldstein et al. 2007). It is not known whether CSEs could also enhance the intracellular anticancer drugs accumulation and cytotoxic activity to overcome multi-drug resistance in tumor cells or not.

In this study, therefore, we encapsulated anti-tumor drug hydroxycamptothecin (HCPT) or fluorescence label rhodamine-123 (RH-123) in CSEs and examined the *in vitro* cytotoxic activity of HCPT-CSEs and the intracellular accumulation of drugs towards a multi-drug resistant cell lines, SGC7901/VCR cells.

2. Investigations and results

2.1. Characteristics of HCPT-CSEs

General physico-chemical properties of HCPT-CSEs such as size analysis, zeta potential, polydispersity, pH values, HCPT

Table: Particle size, zeta potential measurements, polydispersity index, HCPT concentration and visual appearance for HCPT- (n = 3)

	Before autoclaving	After autoclaving
Particle size (nm)	216.70 ± 7.01	221.77 ± 7.88
Polydispersity index	0.18 ± 0.04	0.20 ± 0.018
Zeta potential (mV)	24.36 ± 4.59	19.67 ± 3.44
pH	6.53 ± 0.015	5.66 ± 0.015
HCPT content (μg/mL)	498.19 ± 5.82	484.63 ± 5.55
Visual appearance	Homogenous	Homogenous

content and physical appearance are given in the Table. As expected, the microfluidizer produced smaller droplets of oil dispersed in water. The surface charge of the droplets of HCPT-CSEs was positive. The mean drug loading of HCPT-SEs was 498 μg/ml and no crystal was subsequently detected by an optical microscope at this loading level. The physico-chemical properties of HCPT-CSEs were not significantly affected after autoclaving (115 °C, 30 min). The transmission electron microscopic images of HCPT-SEs revealed their similar spherical shape (Fig. 1). The size from TEM micrographs agreed well with the measurement given by light scattering.

2.2. Cytotoxic activity of HCPT-SEs on sensitive or MDR tumor cells

In order to determine the efficacy of HCPT-CSEs, we investigated the cytotoxic activity of HCPT-CSEs in drug-sensitive SGC7901 cells. The MTT measurement proved that both HCPT-CSEs and HCPT-I were cytotoxic against SGC7901 cells. HCPT-CSEs showed higher antiproliferative activity than HCPT-I (Fig. 2A). IC_{50} values of HCPT-CSEs were 6.7-fold lower than HCPT-I (Fig. 2C).

We investigated the therapeutic efficacy of HCPT treatments in SGC7901/VCR cells which over express P-gp (Fig. 3) and resist to HCPT. As can be seen from Fig. 2B, treatment with various concentrations (0.025–1.0 μg/ml), HCPT-I had no significant effect on the viability of cells. Addition of verapamil, a P-gp inhibitor, resulted in the reversal of drug resistance. Treatment with HCPT-CSEs had significant antiproliferative activity and low IC_{50} value (Fig. 2C), suggesting that HCPT-CSEs overcome drug resistance of this cell line. Though IC_{50} values of HCPT-CSEs were lower than HCPT-I+verapamil to about 2.7-fold (Fig. 2C), antiproliferative activity of HCPT-CSEs to SGC7901/VCR cells lowered as compared to SGC7901 cells.

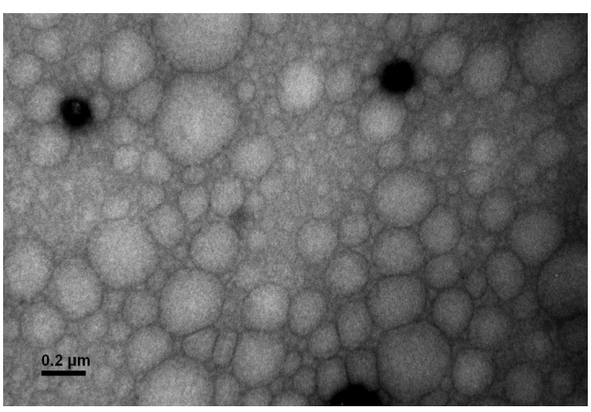
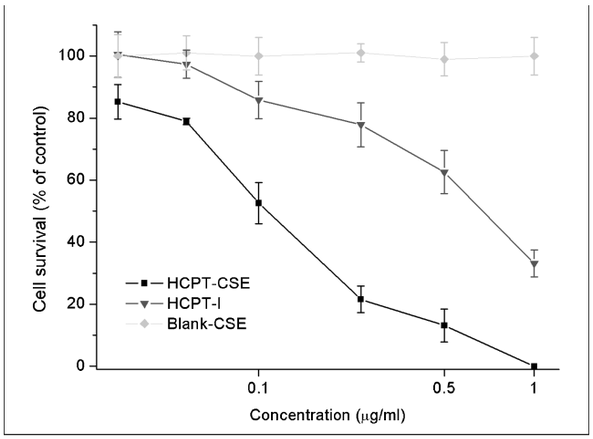


Fig. 1: Transmission electron microscope photograph of HCPT-CSEs

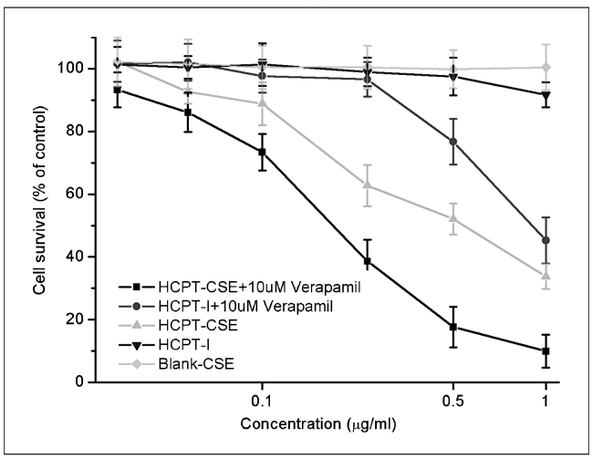
Addition of verapamil slightly enhanced the antiproliferative activity of HCPT-CSEs in SGC7901/VCR cells.

2.3. Accumulation of HCPT in cells

Intracellular accumulation of HCPT was determined following incubation of SGC7901 cells or SGC7901/VCR cells with



(A)



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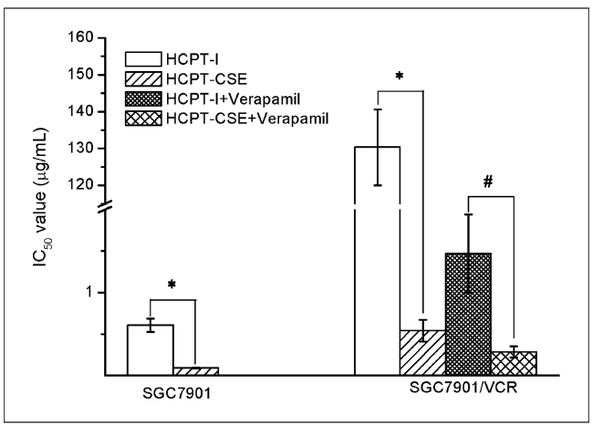
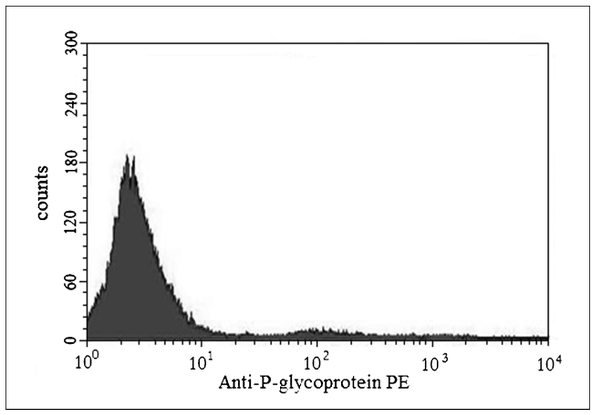
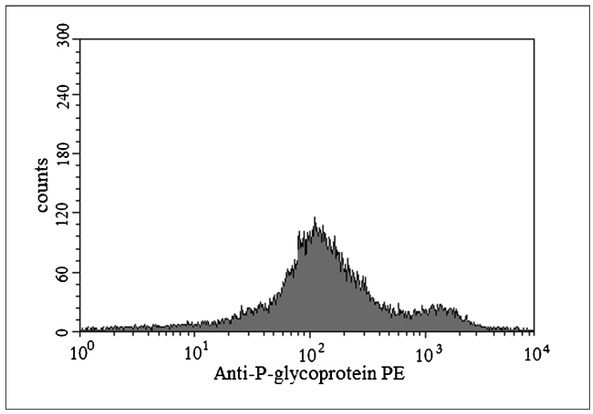


(C)

Fig. 2: Antiproliferative activity of HCPT-I and HCPT-CSEs in SGC7901 cells (A) and SGC7901/VCR cells (B). IC_{50} values (C) of HCPT preparations. Data as mean ± S.D., $n = 4$. $^{*}p < 0.01$ indicates significant differences between HCPT-I and HCPT-CSEs in SGC7901 cells or SGC7901/VCR cells⌊ $^{\#}p < 0.01$ indicates significant differences between HCPT-I and HCPT-CSEs in the presence 10 μM of verapamil towards SGC7901/VCR cells



(A)



(B)

Fig. 3: Flow cytometry analysis of expression of P-glycoprotein in SGC7901 cells (A) and SGC7901/VCR cells (B)

HCPT-CSEs or HCPT-I in the presence or absence of verapamil (Fig. 4). After exposure to HCPT-I, the amount of intracellular HCPT was 8.57 ± 0.10 (ng/10^7 cells) and 3.88 ± 0.20 (ng/10^7 cells) for SGC7901 cells and SGC7901/VCR cells, respectively. It is attracting the HCPT-CSEs resulted in higher drug accumulation which was 6-fold higher than HCPT-I in SGC7901 cells and 8.2-fold higher than in SGC7901/VCR cells. The intracellular uptake of HCPT was also enhanced with addition of verapamil.

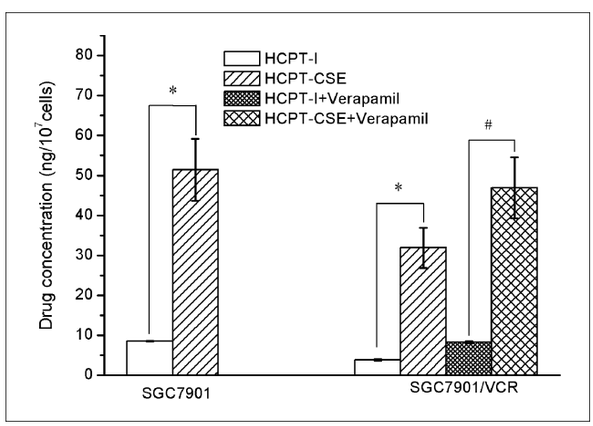


Fig. 4: Intracellular drug accumulation in SGC7901 cells and SGC7901/VCR cells after incubation with 1.0 μg/mL HCPT-CSEs or HCPT-I in the presence or without 10 μM of verapamil for 4 h. Results are expressed as the amount of drug/10^7 cells. *$p < 0.01$ indicates significant differences between HCPT-I and HCPT-CSEs in SGC7901 cells or SGC7901/VCR cells⌊ #$p < 0.01$ indicates significant differences between HCPT-I and HCPT-CSEs in the presence 10 μM of verapamil towards SGC7901/VCR cells

2.4. Accumulation of RH-123 in cells

We have found that significantly higher accumulation of RH-123 in SGC7901/VCR cells was observed in cells treated with verapamil as compared to non-treated cells (Fig. 5), evidently, due to the inhibition of P-gp efflux by verapamil. However, when the same cells were incubated with RH-123-CSEs, the presence or absence of verapamil slightly influenced RH-123 accumulation in the cells. In absence of verapamil, we have found a significantly higher accumulation of RH-123 in cells treated with RH-123-CSEs as compared to RH-123 solution-treated cells.

3. Discussion

The positive charge of submicron emulsions originates from cationic lipids, polymers, and surfactants, such as stearylamine, oleylamine, chitosan, cetyltrimethylammonium bromide, and so on (Shi and Benita 2000). In the current study, stearylamine was used to achieve a positive charge of oil droplets. To improve the stability of HCPT-CSEs, Lipoid E80, Poloxamer 188 and stearylamine which were incorporated in HCPT-CSE, this might enhance the mechanical strength of the interfacial film formed around the oil droplets, and even more, the ionic interactions of the ingredients could create an steric barrier at the oil–water interface (Shi and Benita 2000; Goldstein et al. 2007).

The cytotoxic activity of HCPT-CSEs against the SGC7901 cells and SGC7901/VCR cells was greater than that of HCPT-I. Treatment with HCPT-I had no significant influence on the viability of SGC7901/VCR cells. Adding to verapamil resulted in the reversal of drug resistance. However, verapamil had a slight effect on antiproliferative activity of HCPT-CSEs. Some studies of *in vitro* cytotoxic activity and intracellular drug uptake indicated that increasing intracellular anticancer drug level was likely to lead to an enhanced antiproliferative activity (Gabr et al. 1997; Lundberg et al. 2003; Shabbits and Mayer 2003). Here we determined the intracellular accumulation of HCPT in SGC7901 cells or SGC7901/VCR cells. The results showed that HCPT-CSEs enhanced the more intracellular level of HCPT than HCPT-I in sensitive and drug resistance tumor cells: 8.2-fold higher than HCPT-I in SGC7901/VCR cells. After exposure to HCPT-I, the amount of intracellular HCPT in SGC7901/VCR cells was lower than that in SGC7901 cells for about 2.4-fold. Adding to verapamil resulted in enhancing amount of intracellular HCPT in SGC7901/VCR cells, which was quite significant to HCPT-I.

RH-123, a fluorescent probe, is a substrate for P-gp and can, therefore, be used as a marker for P-gp activity in MDR tumor cells (Dabholkar et al. 2006). On the other hand, verapamil is known to be an inhibitor of P-gp and thus prevents the efflux of P-gp substrates from the cells. In order to future confirm CSEs overcoming P-gp-mediated MDR of tumor cells, we prepared RH-123-CSEs and determined the intracellular accumulation of RH-123 in SGC7901/VCR cells by fluorescence microscopy and flow cytometry. The results agreed with the intracellular accumulation of HCPT.

Cellular uptake of SEs is mediated through energy-dependent endocytosis (Nielsen et al. 2004; Goldstein et al. 2007). The electrostatic mechanism results in a prolonged residence time of CSEs on the negatively charged moieties on the cell surfaces (Shi and Benita 2000; Goldstein et al. 2007). Thus, internalization of CSEs is moderately enhanced, resulting in higher cellular uptake of drugs in oil droplets of CSEs. Due to positive charge

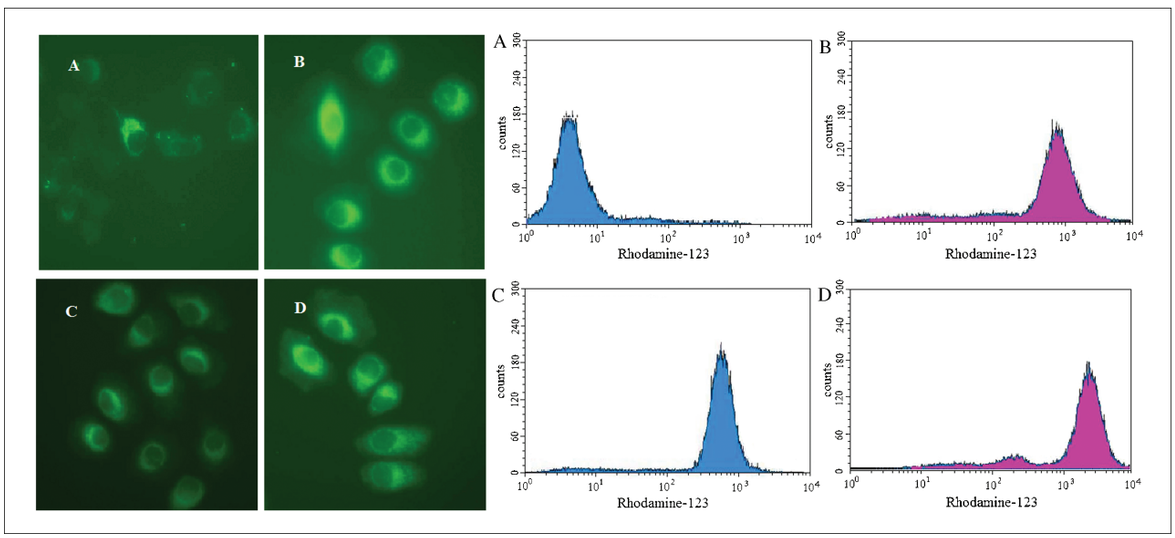


Fig. 5: The effect of CSEs incorporation on P-gp-mediated efflux of RH-123. Fluorescence microscopy (left) and flow cytometry analysis (right) of RH-123 accumulation in SGC7901/VCR cells after incubation with RH-123 solution (A and B) and RH-123-CSEs (C and D) in the absence (A and C) and in the presence (B and D) of verapamil

on surface of drug delivery carrier, cationic delivery carriers can rapidly escape the endo-lysosmal and enter the cytoplasm to keep away from membrane (Zelphati and Szoka 1996a, b; Sonawane et al. 2003). CESs act as intracellular drug depots, release drug in the near nuclear, this decreased P-gp-mediated drug efflux. From this point of view, our data suggested CSEs overcome multidrug resistance in tumor cells of over-expression P-gp by electrostatic-mediated endocytosis.

4. Experimental

4.1. Materials

Hydroxycamptothecin (HCPT, purity > 98.6%) was provided by China Aroma Chemical Co., Ltd (Hangzhou, Zhejiang, China). HCPT-I was purchased from Harbin Sanctity Pharmaceutical Co., Ltd (Harbin, Heilongjiang, China). Acetonitrile and methanol of HPLC grade were obtained from Siyou Chemical Reagent CO., Ltd (Tianjin, China). RPMI1640 and trypsin were purchased from Genom BioMed Technology Inc (Hangzhou, Zhejiang, China). Fetal bovine serum was purchased from Beijing Yuan Heng Sheng Ma BioMed Technology Inc (Beijing, China). Lipoid E80 was purchased from Lipoid GmbH (Ludwigshafen, Germany). Soybean oil was purchased from Tieling Beiya Pharmaceutical Oil Co., Ltd (Liaoning, China). Poloxamer188 was purchased from BASF (Germany). Dimethyl-2,5-diphenyltetrazolium bromide, stearylamine and rhodamine-123 (RH-123) were purchased from Sigma chemical Co. Anti–P-glycoprotein PE was purchased from Becton Dickinson Immunocytometry Systems. All other chemicals were of analytical grade.

4.2. Preparation of cationic submicron emulsions

HCPT-CSEs, which contain HCPT (0.05%, w/v), soybean oil (10%, w/v) as oily phase and Lipoid E80 as emulsifier (2.0%, w/v), were prepared according to the previously described original (Klang et al. 1994). Briefly, Poloxamer 188 (0.5%, w/v) and glycerol (22.5%, w/v) were dissolved in the aqueous. HCPT, soybean oil, Lipoid E80, α-tocopherol and Stearylamine were dissolved in a mixed organic solvent of methanol and chloroform (1:2, v/v) which was then removed by the rotary evaporator under vacuum as oil phase. Both phases were heated separately to 70 °C, and then the two phases were mixed and emulsified by a homogenizer (FJ-200, Shanghai Specimen and Model Factory, Shanghai, China) at 12000 rpm for 5 min. The pH of the crude emulsion was adjusted to 6.5 by citric acid (0.1 M). The emulsion was then passed though Microfluidizer (M-110L, Microfluidics, USA) at 10000 PSI for 4 discrete volume cycles to produce a fine emulsion. RH-123-CSE was prepared in a similar manner by dissolving RH-123 in oil phase.

4.3. Characteristics of HCPT-SEs

Droplet size, polydispersity index and zeta potential were measured by a Malvern Zetasizer Nano-ZS90 (Malvern instruments, UK). HCPT-CSEs were also evaluated for size by a transmission electron microscope (JEM-1200EX, JOEL, TOKYO, JAPAN). A pH meter (PHS-3C Shanghai REX Instrument Factory, China) was used for the determination of the pH value of CSEs at room temperature (21 °C ± 2). The HCPT was measured by a HPLC method.

4.4. Cell culture experiment

A vincristine (VCR)-induced MDR human gastric cancer cell line, SGC7901/VCR cells, and SGC7901 cells (sensitive human gastric cancer cells) were obtained from Basic Medical School of Zhengzhou University (Zhengzhou, Henan, China). SGC7901/VCR cells were grown in RPMI1640 supplemented with 10% (v/v) fetal bovine serum and VCR (at a final concentration of 0.5 mg/L). Cells were incubated in a humidified atmosphere at 37 °C gassed with 5% CO_2 in air, and subcultured every 2 days with 0.25% trypsin.

4.5. Detection of the expression of P-glycoprotein

The expression of P-glycoprotein in MDR tumor cells was detected by immunofluorescence (Chan et al. 1997). Briefly, SGC7901/VCR cells or SGC7901 cells of 70–80% confluence were washed twice with PBS. Twenty microlitres of anti–P-glycoprotein PE was added and mixed thoroughly, then incubated for 30 min in the dark at 25 °C. Cells were then washed twice with sterile PBS and detached by trypsinization. After centrifugation, they were resuspended in sterile PBS. Then the samples were analyzed by a BD FACSCalibur flow cytometry (BD Biosciences, USA) with 3 000 to 10 000 cells measured in each sample.

4.6. Cytotoxic activity studies

The *in vitro* cytotoxic effects of the preparations was measured by a proliferation assay utilizing tetrazolium dye, MTT (Zhao et al. 2007). In brief, cells (SGC7901 cells or SGC7901/VCR cells) were seeded in 96-well plates at a seeding density of 5000 cells/well/0.1 ml medium, and allowed to attach overnight. Cells were then treated with medium containing HCPT-CSEs or HCPT-Injection (HCPT-I), in the presence or absence of 10 μM verapamil. After 72 h, the medium was replaced by fresh medium and the cells were incubated for 4 h with 5 mg/ml MTT, and then the media were replaced with 150 μl DMSO. The optical densities at 492 nm were determined by a micro-plate reader (SUNRISE TECAN, Austria). Results were presented as percentage cytotoxic activity compared to controls.

4.7. Intracellular HCPT accumulation

Intracellular accumulation of HCPT was determined following incubation of SGC7901 cells or SGC7901/VCR cells with HCPT-CSEs or HCPT-I in the presence or absence of verapamil. For determining the intracellular HCPT amount, Cells were seeded 1×10^7 cells/bottle and allowed to attach for 12 h. The cells were incubated with the according agents for 4 h, the incubation was stopped by washing the cells thrice with ice-cold PBS (pH 7.4) and detached by trypsinization. After centrifugation, they were resuspended in sterile PBS. The HCPT amount in cells were quantified by a reversed-phase

HPLC assay described previously (Zhao et al. 2006). Cells were lysed over ice by sonication (6/8 s, 200 W, 20 times). Twenty-microlitre phosphoric acid was added to 1.0 ml aliquots of cell lysis solution (10^7 cells/ml) for HCPT in the closed lactone ring form and vortexed for 3 min. The mixture was extracted with ethyl acetate, vortexed for 3 min. The mixture was extracted with 5.0 ml of ethyl acetate, vortexed for 5 min then centrifuged for 15 min at 3 500 rpm. Collected the upper organic layer and evaporated it to dryness with N_2 at 35 °C. The residue was reconstituted in 100 μl methanol, vortexed for 3 min. After centrifugation, the extracted HCPT was determined by HPLC (Agilent 1100) at an excitation wavelength of 382 nm and an emission wavelength of 528 nm, respectively.

4.8. Intracellular RH-123 accumulation studied by fluorescence microscopy

SGC7901/VCR cells were grown in six-well tissue culture plates. After the cells reached a confluence of 70–80%, they were washed twice with PBS buffer. RH-123 solution was added to each well so that they contained 5.0 μM of RH-123 in 1% methanol in PBS buffer or 5.0 μM of RH-123 encapsulated in CSEs in PBS buffer with or without 10 μM of verapamil. After the incubation for 120 min at 37 °C, 5% CO_2, cells were washed with the PBS buffer and further incubated with or without 10 μM verapamil for another 45 min at 37 °C, 5% CO_2. The cells were washed thrice with sterile PBS, and observed with Olympus IX-71 fluorescence microscope (Olympus, Japan) under Rhodamine/FITC filter.

4.9. Intracellular RH-123 accumulation studied by flow cytometry analysis

SGC7901/VCR cells were grown in six-well tissue culture plates. After the cells reached a confluence of 70–80%, they were washed twice with the PBS buffer. The cells were exposed to 5.0 μM RH-123 in 1% methanol in the PBS buffer or encapsulated in CSEs in PBS buffer with or without 10 μM of verapamil hydrochloride. After the incubation for 120 min at 37 °C, 5% CO_2, cells were washed with the PBS buffer and incubated with or without 10 μM verapamil for 2 h at 37 °C, 5% CO_2. The cells were washed twice with sterile PBS and detached by trypsinization. After centrifugation, they were resuspended in sterile PBS. Then the samples were analyzed by flow cytometry.

4.10. Statistical analysis

The statistical analysis of experimental data was carried out using the Student's t-test. Differences were considered significant at $p < 0.05$. Data are presented as mean ± S.D.

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References

Barraud L, Merle P, Soma E, Lefrancois L, Guerret S, Chevallier M, Dubernet C, Couvreur P, Trepo C, Vitvitski L (2005) Increase of doxorubicin sensitivity by doxorubicin-loading into nanoparticles for hepatocellular carcinoma cells *in vitro* and *in vivo*. J Hepatol 42: 736–743.

Batrakova EV, Li S, Miller DW, Kabanov AV (1999) Pluronic P85 increases permeability of a broad spectrum of drugs in polarized BBMEC and Caco-2 cell monolayers. Pharm Res 16: 1366–1372.

Breuninger LM, Paul S, Gaughan K, Miki T, Chan A, Aaronson SA, Kruh GD (1995) Expression of multidrug resistance-associated protein in NIH/3T3 cells confers multidrug resistance associated with increased drug efflux and altered intracellular drug distribution. Cancer Res 55: 5342–5347.

Chan HS, Haddad G, Zheng L, Bradley G, Dalton WS, Ling V (1997) Sensitive immunofluorescence detection of the expression of P-glycoprotein in malignant cells. Cytometry 29: 65–75.

Chavanpatil MD, Patil Y, Panyam J (2006) Susceptibility of nanoparticle-encapsulated paclitaxel to P-glycoprotein-mediated drug efflux. Int J Pharm 320: 150–156.

Dabholkar RD, Sawant RM, Mongayt DA, Devarajan PV, Torchilin VP (2006) Polyethylene glycol-phosphatidylethanolamine conjugate (PEG-PE)-based mixed micelles: some properties, loading with paclitaxel, and modulation of P-glycoprotein-mediated efflux. Int J Pharm 315: 148–157.

Deffie AM, Batra JK, Goldenberg GJ (1989) Direct correlation between DNA topoisomerase II activity and cytotoxicity in adriamycin-sensitive and -resistant P388 leukemia cell lines. Cancer Res 49: 58–62.

Gabr A, Kuin A, Aalders M, El-Gawly H, Smets LA (1997) Cellular pharmacokinetics and cytotoxicity of camptothecin and topotecan at normal and acidic pH. Cancer Res 57: 4811–4816.

Garcion E, Lamprech, A, Heurtault B, Paillard A, Aubert-Pouessel A, Denizot B, Menei P, Benoit JP (2006) A new generation of anticancer, drug-loaded, colloidal vectors reverses multidrug resistance in glioma and reduces tumor progression in rats. Mol Cancer Ther 5: 1710–1722.

Gatouillat G, Odot J, Balasse E, Nicolau C, Tosi PF, Madoulet C (2007) Immunization with liposome-anchored pegylated peptides modulates doxorubicin sensitivity in P-glycoprotein-expressing P388 cells. Cancer Lett 257:165–171.

Goldstein D, Sader O, Benita S (2007) Influence of oil droplet surface charge on the performance of antibody–emulsion conjugates. Biomed Pharmacother 61: 97–103.

Gottesman MM, Fojo T, Bates SE (2002) Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer 2: 48–58.

Kirkin V, Joos S, Zornig M (2004) The role of Bcl-2 family members in tumorigenesis. Biochim Biophys Acta 1644: 229–249.

Klang SH, Frucht-Pery J, Hoffman A, Benita S (1994) Physicochemical characterization and acute toxicity evaluation of a positively-charged submicron emulsion vehicle. J Pharm Pharmacol 46: 986–993.

Krishna R, Mayer LD (1999) The use of liposomal anticancer agents to determine the roles of drug pharmacodistribution and P-glycoprotein (PGP) blockade in overcoming multidrug resistance (MDR). Anticancer Res 19: 2885–2891.

Krishnamachary N, Center MS (1993) The MRP gene associated with a non-P-glycoprotein multidrug resistance encodes a 190-kDa membrane bound glycoprotein. Cancer Res 53: 3658–3661.

Lee ES, Na K, Bae YH (2005) Doxorubicin loaded pH-sensitive polymeric micelles for reversal of resistant MCF-7 tumor. J Control Release 103: 405–418.

Lucci A, Han TY, Liu YY, Giuliano AE, Cabot MC (1999) Modification of ceramide metabolism increases cancer cell sensitivity to cytotoxics. Int J Oncol 15: 541–546.

Lundberg BB, Risovic V, Ramaswamy M, Wasan KM (2003) A lipophilic paclitaxel derivative incorporated in a lipid emulsion for parenteral administration. J Control Release 86: 93–100.

Mayer LD, Shabbits JA (2001) The role for liposomal drug delivery in molecular and pharmacological strategies to overcome multidrug resistance. Cancer Metastasis Rev 20: 87–93.

Na K (2007) pH-sensitive polymeric micelles for the effective delivery of anti-cancer drug. Korean J Gastroenterol 49: 314–319.

Nielsen HM, Aemisegger C, Burmeister G, Schuchter U, Gander B (2004) Effect of oil-in-water emulsions on 5-aminolevulinic acid uptake and metabolism to PpIX in cultured MCF-7 cells. Pharm Res 21: 2253–2260.

Nobili S, Landini I, Giglioni B, Mini E (2006) Pharmacological strategies for overcoming multidrug resistance. Curr Drug Targets 7: 861–879.

Seid CA, Fidler IJ, Clyne RK, Earnest LE, Fan D (1991) Overcoming murine tumor cell resistance to vinblastine by presentation of the drug in multilamellar liposomes consisting of phosphatidylcholine and phosphatidylserine. Sel Cancer Ther 7: 103–112.

Shabbits JA, Mayer LD (2003) Intracellular delivery of ceramide lipids via liposomes enhances apoptosis *in vitro*. Biochim Biophys Acta 1612: 98–106.

Shabbits JA, Krishna R, Mayer LD (2001) Molecular and pharmacological strategies to overcome multidrug resistance. Expert Rev Anticancer Ther 1: 585–594.

Shi CY, Benita S (2000) Enhanced absorption and drug targeting by positively charged submicron emulsions. Drug Dev Res 50: 476–486.

Soma CE, Dubernet C, Bentolila D, Benita S, Couvreur P (2000) Reversion of multidrug resistance by co-encapsulation of doxorubicin and cyclosporin A in polyalkylcyanoacrylate nanoparticles. Biomaterials 21: 1–7.

Sonawane ND, Szoka FC Jr, Verkman AS (2003) Chloride accumulation and swelling in endosomes enhances DNA transfer by polyamine-DNA polyplexes. J Biol Chem 278: 44826–4431.

Thierry AR, Vige D, Coughlin SS, Belli JA, Dritschilo A, Rahman A (1993) Modulation of doxorubicin resistance in multidrug-resistant cells by liposomes. Faseb J 7: 572–579.

Vauthier C, Dubernet C, Chauvierre C, Brigger I, Couvreur P (2003) Drug delivery to resistant tumors: the potential of poly(alkyl cyanoacrylate) nanoparticles. J Control Release 93: 151–160.

Viktorsson K, De Petris L, Lewensohn R (2005) The role of p53 in treatment responses of lung cancer. Biochem Biophys Res Commun 331: 868–880.

Zelphati O, Szoka FC Jr (1996a) Mechanism of oligonucleotide release from cationic liposomes. Proc Natl Acad Sci U S A 93: 11493–11498.

Zelphati O, Szoka FC Jr (1996b) Intracellular distribution and mechanism of delivery of oligonucleotides mediated by cationic lipids. Pharm Res 13: 1367–1372.

Zhang K Mack P, Wong KP (1998) Glutathione-related mechanisms in cellular resistance to anticancer drugs. Int J Oncol 12: 871–882.

Zhao YX, Gao JQ, Qiao HL, Chen HL, Liang WQ (2006) Development and validation of a sensitive reversed-phase HPLC method to determine intracellular accumulation of hydroxycamptothecin. J Pharm Biomed Anal 41: 1007–1010.

Zhao YX, Gao JQ, Sun XY, Chen HL, Wu LM, Liang WQ (2007) Enhanced nuclear delivery and cytotoxic activity of hydroxycamptothecin using o/w emulsions. J Pharm Pharm Sci 10: 61–70.