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Research Article

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Study of co-encapsulated doxorubicin and curcumin poly(butyl cyanoacrylate) nanoparticles and reversion of multidrug resistance in MCF-7/ADR cell line

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ABSTRACT

Co-encapsulated DOX(doxorubicin) and CUR(curcumin) in PBCA-NPs(polybutylcyanoacrylate nanoparticles) were prepared with emulsion polymerization in an attempt to show the improved growth inhibition efficacy in a resistant cell culture line. The mean particle size and the zeta potential of DOX-CUR-PBCA-NPs were 133 ± 5.34nm in diameter and +32.23 ± 4.56 mV, respectively. The entrapment efficiencies of DOX and CUR were 49.98 ± 3.32% and 94.52 ± 3.14% respectively. Anticancer activities and reversal efficacies of the formulations and various combination approaches were assessed using MTT assay and Western Blotting. The results showed that the dual-agent loaded PBCA-NPs system had the similar cytotoxicity to co-administration of two single-agent loaded PBCA-NPs (DOX-PBCA-NPs + CUR-PBCA-NPs), which was slightly higher than that of the free drug combination (DOX-CUR) and one free drug / another agent loaded PBCA-NPs combination (DOX+CUR-PBCA-NPs or CUR+DOX-PBCA-NPs). The simultaneous administration of DOX and CUR could achieve the highest efficacy in terms of reversing multidrug-resistantance(MDR), and down-regulating P-gp in MCF-7/ADR cell lines. MDR reversal can be enhanced by treated combination of encapsulated cytotoxic drugs and reversal agents. Co-encapsulation of anticancer drug DOX and reversal agent CUR can be more effective in reversing multi-drug resistance than the other formulations and might cause lower normal tissue drug toxicity and fewer drug-drug interactions.

Keywords: Poly(butyl cyanoacrylate) nanoparticles, Curcumin, Doxorubicin, Adriamycin-resistant human breast carcinoma cell line, Multidrug resistance

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INTRODUCTION

Cancer is one of the greatest threats to human beings. According to a 2005 WHO report, deaths from cancer account for about 13% of the world's 58 million annual deaths. At present, chemotherapy, surgery and radiotherapy are three major treatments for cancer. But, as most chemotherapy drugs are not precisely targeted at cancer cells, many patients often give up their treatment once they find severe side effects elicited by these drugs[1-4]. DOX (doxorubicin), a broad spectrum antitumor drug, can inhibit the synthesis of DNA and RNA, thus it can be used to kill tumor cells of different growth cycle. Because of its cytotoxic effect on tumor cells, DOX is mainly used with other anticancer drugs in the treatment of lymphoma and acute leukemia. And it also exhibits a certain positive effects on curing breast cancer, liver cancer, lung cancer, bladder cancer and other various types of cancers [5, 6]. In

clinical practice, MDR (mu1tidrug resistance) is the main reason behind the failure of chemotherapy. Therefore, how to overcome multidrug resistance is of great importance to the efficacy and the prognosis of chemotherapy.

CUR (Curcumin), which is known as a multifunctional medicine: an anti-cancer, anti-angiogenic, anti-atherosclerosis anti-oxidant, and anti-inflammatory agent, has been called by National Cancer Institute (America) as a universal cancer prophylactic agent [7]. Additionally, with low toxicity and cheap price, this chemical can also be used to improve chemosensitivity and MDR reversal.

CUR demonstrates synergistic inhibitory effect on various tumor cells, when used with either cell cycle nonspecific or specific agents. Furthermore, CUR can also reduce the toxicity of chemotherapy drugs, and enhance curative effects on tumor cells.

In this study, co-encapsulated DOX and CUR in Poly (butyl cyanoacrylate) nanoparticles (PBCA-NPs) were prepared with emulsion polymerization. DOX-CUR-PBCA-NPs were characterized by FT-IR and GPC, and reversal effects on MDR in human breast cancer cell line MCF7/ADR were evaluated by MTT and Western blotting.

EXPERIMENTAL SECTION

2.1. Materials

Butyl cyanoacrylate (BCA) monomer was obtained from Guangzhou Baiyun Medical Adhesive (China). Chitosan (Deacetylation≥85%) was supplied by Sigma (USA). CUR （AR）was purchased from Sinopharm Chemical Reagent (China) and DOX was from National Institutes for Food and Drug Control (China). Methyl thiazolyl tetrazolium(MTT) was from Hefei Bomei Biotechnology (China). Bradford Protein Assay Kit was purchased from Betotime Institute of Biotechnology (China). P-glycoprotein (MDR1) was supplied by Wuhan Boster Biotechnology (China). Anti-β-actin rabbit polyclonal antibody was from Abgent (USA). Horseradish peroxidase conjugated Donkey anti-rabbit IgG was obtained from Santa Cruz (USA). ECL (Electro Chemical Luminescence) substrate was from Pierce (USA).

2.2. Cell Line Culture

Cell cultures were maintained in a humidified 37℃incubator with a 5% carbon dioxide in air atmosphere. MCF-7 and MCF-7/ADR resistant cells were grown in suspension in RPMI 1640 medium (Gibco, France), supplemented with 10% foetal calf serum (Gibco, France). 1000ng/mL DOX were added to the medium of MCF-7/ADR and removed one week before the experiment.

2.2. Methods

2.3.1. Preparation of DOX-CUR-PBCA-NPs

DOX-CUR-PBCA-NPs were prepared by emulsion polymerization process, which was subject to the following procedure: CUR, DOX and 0.1% chitosan were dissolved in anhydrous ethanol, distilled water, and 10 mL hydrochloric acid solution, respectively. Then CUR solution and DOX solution were added to chitosan solution. Afterwards, α-BCA was introduced dropwise during a 6h stirring. After stirring, the suspension was neutralized to pH=4 using NaOH. DOX-CUR-PBCA-NPs were obtained following centrifugalization(16000 rpm for 30 min).They were washed three times by distilled water and dried. DOX-PBCA-NPs and CUR-PBCA-NPs were prepared with the same procedure.

2.3.2. Characterization of DOX-CUR-PBCA-NPs

2.3.2.1. Particle Size and Zeta Potential Measurement

The sizes of the NPs were determined by transmission electron microscopy (TEM).To determine the particle size, three samples of DOX-CUR-PBCA-NPs suspension were taken in this test. The 1st sample was dropped on a carbon-coated cooper wire and then added with phosphotungstic acid (2%, pH 6.2) for negative staining. After natural air-dry, it was observed by transmission electron microscope.

Mean particle size was determined by Particles Size Analyzer (Zetasizer Nano system) after the 2nd sample was diluted with double distilled water.

Zeta potential was examined by Zeta Potential Analyzer (Zetasizer Nano system) after the 3rd sample was diluted with 10 mmol/l NaCl.

2.3.2.2. Entrapment and Loading Efficiency Measurement

DOX-CUR-PBCA-NPs suspension was centrifuged at 16000 rpm for 0.5h. Then it was washed by distilled water four times and the supernatant was analyzed by HPLC (Shimadzu LC10AT, Japan) with a UV detector. The content of CUR and DOX was determined by HPLC at 423nm and 232.5nm, respectively. To assess chemical integrity during the preparation process, DOX-CUR-PBCA-NPs were subject to the following HPLC procedure: The sample was transported through a column (4.6mm×250 mm, 5μm, Hypersil BDS C18, Dalian) by a mobile phase composed of 70% methanol and 30% acetic acid (2%). The solvent flow rate was 1.0 ml/min and the column was kept at 30℃. Entrapment and loading efficiencies (%) were expressed as follows:

Entrapment Efficiency=100 %×(total CUR/total DOX – the CUR in supernatant/the DOX in supernatant)/ (total CUR/ total DOX)

Loading Efficiency= 100 %×(total CUR/ total DOX – the CUR in supernatant/the DOX in supernatant)/ (total DOX-CUR-PBCA-NPs)

2.3.2.3. FT-IR Analysis

Samples of empty PBCA-NPs, DOX-CUR-PBCA-NPs, mixture of free CUR powder and free DOX powder, and mixture of empty PBCA-NPs, free CUR powder and free DOX powder were measured with KBr pellet by FTIR. Spectra were recorded using a Bruker spectrometer. All the spectra were taken from400~4000 cm-1.

2.3.2.4． GPC Analysis

The molecular weights, including Mn (number-average molecular weight), Mw (weight-average molecular weight), Mp (peak molecular weight), Mz (viscosity-average molecular weight), and PI or Mw/Mn (relative molecular mass distribution index), of PBCA-NPs, DOX-PBCA-NPs, CUR-PBCA-NPs, DOX-CUR-PBCA-NPs were characterized by gel permeation chromatography.

After drying, the samples were dissolved with THF and concentrated. Then 20μL of each sample was filtered through a 0.2μm PTFE membrane. The GPC analysis was performed at 38℃ in THF solvent at a flow rate of 1.0ml/min−1 using a GPC equipped with Waters 2414 refractive index detector and a column set of three 300mm×7.8 mm columns(HR 1. 3. 4.). Molecular weights were calculated relative to polystyrene standards and standards of 462000, 186000, 114000, 43700, 18600, 9650, 6520, 2950, 461 Da were used for column calibration.

2.3.3. MTT Assay

The cells were plated into 96-well microtiter plates (200 μL each) at a cellular density of 4×10^3 cells/well. The wells, which contained 200 μL medium but no cells, were used as blank control group ,and the wells ,which contained 200 μL cell suspension but no drugs, were used as negative control group .The wells contained 200 μL fixed concentration(0.2 μg/ ml CUR and 0.12 μg/ml DOX) suspension of PBCA-NPs, CUR-PBCA-NPs, DOX-PBCA-NPs, DOX-CUR-PBCA-NPs ,the mixture of single-loading NPs(CUR-PBCA-NPs + DOX-PBCA-NPs) and the mixture of free powders(CUR + DOX) were used as experiment groups. Each dose group took 6 wells. Cells were incubated for 48h. After the incubation, the medium was removed and the cells were washed three times with 1 × PBS solution. Then 200 μL new medium and 20 μL MTT (5mg/mL) were added to each well for 4h incubation. After that, the supernatant of each well was removed. Then each well was added with 150μL DMSO and went through 10-minute oscillation (room temperature).Afterwards, each well was analyzed at 570 nm by Multiskan Spectrum (Electro Thermo, USA). The inhibition rate of the treated cells was calculated in regard to the ultraviolet absorbance value of the test wells and the control wells.

Inhibition rate (%) = 100% × (optical density of the blank control group − optical density of the experiment group)/ optical density of the blank control group

2.3.4. Western Blotting Assay

MCF-7/ADR resistant cell lines were plated into microtiter plates (100mm each) at different cellular density and treated with suspensions (each containing 0.2 μg/ml CUR and 0.12 μg/ml DOX) of PBCA-NPs, CUR-PBCA-NPs, DOX-PBCA-NPs, DOX-CUR-PBCA-NPs for 48h. Afterwards, the cells were collected, and centrifuged at 1,000 rpm for 5 min. Then they were washed in 1 × PBS (4℃) and lysed in EBC buffer which contained 50 mM Tris (pH 7.5), 150 mM NaCl, 0.5% Nonidet P-40 and protease inhibitors. After ultrasonication and 10min-centrifuge (14,000rpm), the whole-cell proteins were extracted from the supernatant .They were stored at -20℃.

Practically, 100μg proteins were separated through SDS-PAGE electrophoresis and transferred to nitrocellulose membranes. The membranes were blocked in 5% nonfat dry milk for 2h and incubated overnight at 4℃ with MDR1 (diluted 1:200) antibody .After washed three times in 1×PBS (5 minute each time), it was incubated with Horseradish peroxidase conjugated Donkey anti-rabbit IgG (diluted 1:1000) for 1h at room temperature. The blots were exposed to Hyper film ECL and β-actin blots were used as loading controls.

2.3.5 Statistic Analysis

The experimental data were processed by SPSS 17.0 software, described in the form of the mean value± standard deviation, and compared through one-way analysis of variance ($P < 0.05$, significant difference; $P < 0.01$, very significant difference).

RESULTS AND DISCUSSION

3.1. Characteristics of Nanoparticles

The transmission electron microscopy of DOX-CUR-PBCA-NPs (Fig.1) indicates that the nanoparticles were spherical in shape, uniform in size and had good dispersity and relatively complete integrity. The particle size was 133 ± 5.34mm in diameter, larger than that of single drug loaded particles.

This observation suggests that during α-BCA polymerization, the free amino groups of DOX increased the polymerization reaction rate as an initiator, and DOX was added to the molecular chains by covalent bond. The presence of Cur hindered DOX to take part in the polymerization process of α-BCA, stopping chain growth and hence leading to a smaller particle size of DOX-CUR-PBCA-NPs.

Zeta potential results indicates that in 10mM NaCl solution, the zeta potential of DOX-CUR-PBCA-NPs were +32.23 ± 4.56 mV. HPLC results shows that the entrapment efficiencies of DOX and CUR were 49.98 ± 3.32% and 94.52 ± 3.14% and loading efficiencies of DOX and CUR were0.619% ± 0.05% and 1.17% ± 0.04%.

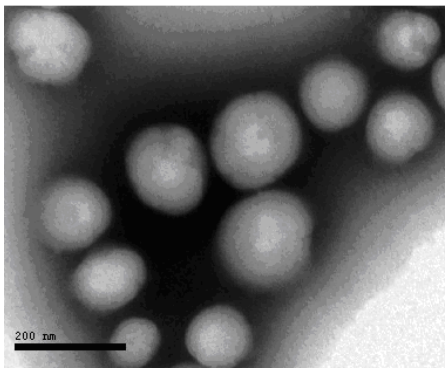

Fig. 1 The transmission electron microscopy of DOX-CUR-PBCA-NPs

3.2 FTIR Analysis

The spectra of different NPs provided a number of spectral details indicating some similarities and variations among them. Fig2(c, d) shows similarities between DOX-CUR-PBCA-NPs and empty PBCA-NPs. The characteristic peaks of cyanogroup of DOX-CUR-PBCA-NPs and empty PBCA-NPs were observed at 2250.91cm-1(Fig.2 c) and 2251.47cm-1(Fig2 d) suggesting that the cyanogroup of α-BCA was a strong electron withdrawing substitution

which didn't take part in the process of anionic emulsion polymerization. The spectra(Fig2,e) of the mixture (empty PBCA-NPs , CUR powder and DOX powder)also exhibits the characteristic peaks of cyanogroup, but meanwhile some characteristic peaks of CUR powder and DOX powder were shown at around 1000~2000 cm^{-1}. For example, the characteristic peaks of carbonyl group on the anthracene ring of DOX, which appeared at 1621cm-1 and 1585.10cm-1 in DOX powder, were present at 1625.44cm-1 and 1589.92cm-1 in the mixture. Similarly, the adsorption peak of phenolic hydroxyl group in free CUR powder (3414.71cm-1) was also appeared in the mixture and the adsorption peak of carbonyl group on the benzene ring of CUR, which showed at 1510.01cm-1 in free CUR powder, was centered at 1514.45cm-1 in the mixture. But none of the characteristics peaks of free CUR powder and free DOX powder above appeared in the spectra of DOX-CUR-PBCA-NPs. This indicates that through the emulsion polymerization process applied in the paper, CUR and DOX were involved in the α-BCA polymerization process, and were encapsulated by PBCA-NPs, instead of adhering to its surface.

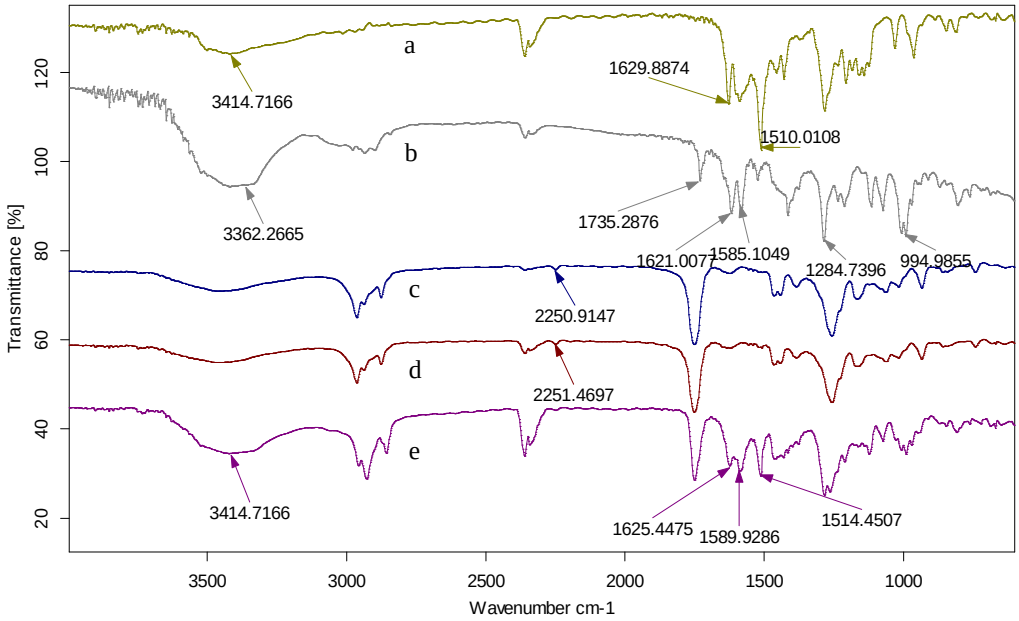

Fig. 2 Fourier-Transform IR (FTIR) spectra of CUR (a), DOX (b), empty PBCA-NPs (c), CUR-DOX-PBCA-NPs (d), physical mixture of free DOX, free CUR and empty PBCA-NPs (e)

3.3 Molecular Weights (GPC Analysis)

The molecular weights, including Mn (number-average molecular weight), Mw (weight-average molecular weight), Mp (peak molecular weight), Mz (viscosity-average molecular weight) ,and PI or Mw/Mn (relative molecular mass distribution index), of PBCA-NPs, DOX-PBCA-NPs, CUR-PBCA-NPs, DOX-CUR-PBCA-NPs were characterized by gel permeation chromatography and calculated relative to polystyrene standards.

The Mn(1742) and the Mw (1995) of CUR-PBCA-NPs as well as the Mn(1612) and the Mw(1998) of PBCA-NPs displayed in Table1 suggests that there was no significant difference between the molecular weights of CUR-PBCA-NPs and PBCA-NPs ($P<0.05$), which confirmed the previous results that the presence of CUR didn't lead to the chain growth of NPs. In contrast, the GPC analysis on DOX-PBCA-NPs shows quite different results. Compared with PBCA-NPs, the Mn (1888) and the Mw(2122) of DOX-PBCA-NPs increased significantly($P<0.01$), which also confirmed the previous result that DOX was involved in the α-BCA polymerization process and contributed to the chain growth. Fig3 also suggests that there was no significant difference between the Mn and the Mw of DOX-CUR-PBCA-NPs and CUR-PBCA-NPs. And this once again demonstrated the previous result that the presence of Cur hindered DOX to take part in the polymerization process of α-BCA, stopping chain growth and hence leading to a smaller particle size.

The GPC chromatographic profiles of PBCA-NPs（A）、CUR-PBCA-NPs（B）、DOX-PBCA-NPs（C）and DOX-CUR-PBCA-NPs（D）in Fig.3 shows an approximately 24min retention and a wide molecular distribution

for all NPs. And the PI in Fig 4 reveals that there was no significant difference between the dispersity of these NPs. In addition, two minor peaks found on the profiles indicate that two smaller molecules may exist. But because the molecular weights of them exceeded the range of the calibration curve, the exact molecular weight could not be determined.

Table 1 Molecular weight distribution of different kinds of PBCA Nps

nanoparticles	M_n	M_W	M_P	M_Z
PBCA-NPs	1612	1988	2071	2370
CUR-PBCA-NPs	1742	1995	2214	2234
DOX-PBCA-NPs	1888	2122	2146	2354
DOX-CUR-PBCA-NPs	1723	1933	2049	2144

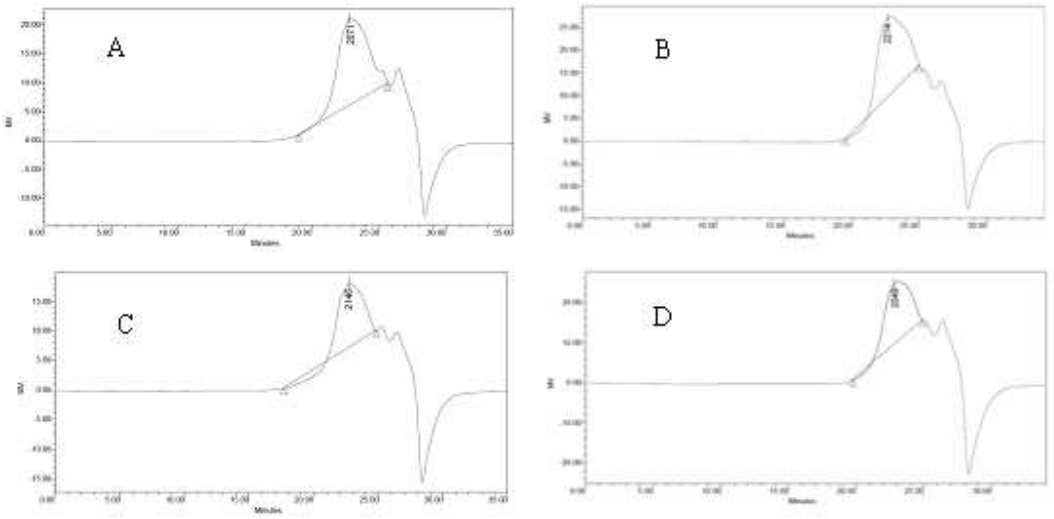

Fig. 3 GPC chromatographic profiles of PBCA-NPs（A）、CUR-PBCA-NPs（B）、DOX-PBCA-NPs（C）and DOX-CUR-PBCA-NPs（D）

3.4 Inhibition of Cell Growth

Table2 shows the growth inhibition effects on MCF-7/ADR resistant cell line by PBCA-NPs (each containing 0.2 μg/ml CUR and 0.12 μg/ml DOX). After 48h incubation at 37℃ in a 5% CO_2 atmosphere, the cell growth inhibitory rate of CUR+DOX only achieved 5.32%, and of empty PBCA-NPs just reached 5.14%. However, under the same condition high cell growth inhibitory rates were observed in CUR-PBCA-NPs, DOX-PBCA-NPs, DOX-CUR-PBCA-NPs, DOX+CUR-PBCA-NPs, CUR+DOX-PBCA-NPs, CUR-PBCA-NPs+DOX-PBCA-NPs. The results shows that the inhibitory rates of DOX-CUR-PBCA-NPs (96.78%) was equivalent to CUR-PBCA-NPs + DOX-PBCA-NPs (96.94%),which were slightly higher than that of CUR+DOX-PBCA-NPs(89.72%) ,DOX+CUR-PBCA-NPs(84.94%), CUR-PBCA-NPs(85.15%), and DOX-PBCA-NPs(89.43%). This makes it clear that when encapsulated in NPs, DOX and CUR could work synergistically in killing drug resistant cell lines and have great potential in reversing MDR in MCF-7/ADR resistant cell line.

Table2 Growth Inhibition on MCF-7/ADR resistant cell line

nanoparticles	inhibitory rates（%）
PBCA-NPs	5.14
CUR+DOX	5.32
CUR-PBCA-NPs	85.15
DOX-PBCA-NPs	89.43
CUR+DOX-PBCA-NPs	89.72
DOX+CUR-PBCA-NPs	84.94
CUR-PBCA-NPs+ DOX-PBCA-NPs	96.94
DOX-CUR-PBCA-NPs	96.78

3.5 Effects of Nanoparticles on MDR1

Breast cancer is a chemosensitive solid tumor and the main reason of unsuccessful chemotherapy is multidrug resistance (MDR).

Fig.4 suggests that there was significant difference between the expression of MDR1 proteins in MCF-7 cells and in MCF-7/ADR cells.

Fig.5 shows the effects of different formulations on MDR1 expression in MCF-7/ADR cells. After 48h incubation, MDR1 expression in MCF-7/ADR cells was significantly down regulated by DOX-CUR-PBCA-NPs and CUR-PBCA-NPs+DOX-PBCA-NPs (each containing 0.2 μg/ml CUR and 0.12 μg/ml DOX). Their reversal efficacies, which were equivalent to each other, were significantly higher than that of CUR+DOX, CUR-PBCA-NPs+DOX, and DOX-PBCA-NPs+CUR. This reveals that DOX-CUR-PBCA-NPs could achieve a much lower expression level of efflux pump protein P-gp in MCF-7/ADR than the single agent loaded PBCA-NPs.

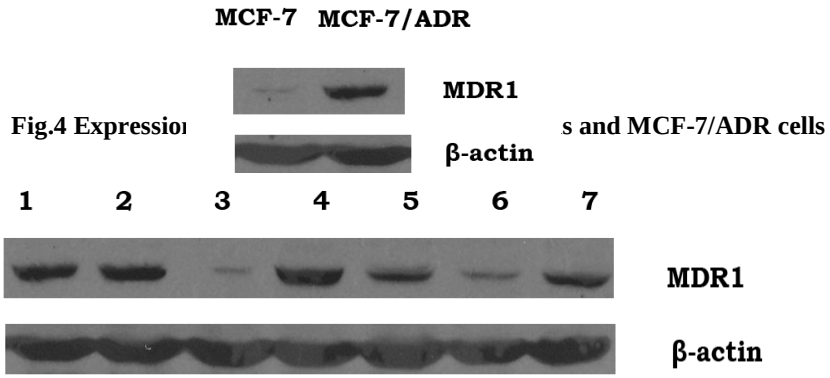

Fig.5 Effects of different Nps on MDR1 expression in MCF-7/ADR cells. 1: control; 2: PBCA-NPs; 3: CUR-DOX-PBCA-NPs; 4: CUR + DOX; 5: CUR-PBCA-NPs + DOX; 6: CUR-PBCA-NPs + DOX-PBCA-NPs; 7: DOX-PBCA-NPs + CUR

Studies on molecular mechanisms of MDR and reversal agents have become popular in recent years. At present, the most investigated mechanisms with known clinical significance are: a) activation of MDR1 and P-gp; b) activation of the enzymes of the glutathione detoxification system; c) mutations of the genes coding for topoisomerases, enhancement of DNA repair; d) activation of multidrug resistance-associated protein MRP [8]. To reverse the above mechanisms, many drugs have been invented. Currently, the main groups of anticancer drugs include : a)verapamil, cyclosporine A ,and their derivatives; b)thiophene compounds ;c)hormones, e.g. , progesterone, megestrol acetate ,etc ;d)anti-malaria and anti-arrhythymic drugs , e.g. , quinine ,quinidine ,etc.

However, as many of these drugs are not initially used as anti-cancer drugs, they often exhibit side effects and instability, and could damage targets in cells. Therefore, only very few of these drugs, such as verapamil and cyclosporin A, can be put into clinical use but their efficacies are not very satisfactory. In order to find better drugs, in recent years scientists have shifted their study to the reversal effect on low toxicity components extracted from traditional Chinese medicine, and have achieved some progress in this field[9].

Compared with other chemical reversal agents, traditional Chinese medicine has a lot of advantages: multiple targets, fewer side effects, and multiple functions (simultaneously killing tumor cells, and adjusting, improving the immune function during reversal process) CUR, the major active ingredient of a common traditional Chinese medicine curcuma, is known as an effective anti-cancer, antiangiogenic, anti-atherosclerosis, anti-oxidant and anti-inflammatory agent [10-16]. Therefore, the reversal effect of CUR on multidrug resistance has become a very popular research topic in recent years.

The current studies and researches on MDR reversal by CUR include: (1) Reversal effect of CUR on membrane transporters mediated tumor multidrug-resistance. Hou et al. [17] demonstrated that curcuma drugs and CUR could down regulate the expression of P-gp and MDR1 in Caco-2 cells. Moreover, Angelini et al [18] found that CUR could increase the concentration of DOX in human sarcoma MES-SA/Dx-5 cells and reverse P-gp-mediated

multidrug resistance. (2) Reversal effect of CUR on enzyme system mediated tumor multidrug-resistance. Andjelkovic et al. [19] showed that CUR could decrease the expression of Topo II and GST in multidrug resistant human non-small cell lung carcinoma cell line (NCI-H460/R). (3) Reversal effect of CUR on apoptosis proteins mediated tumor multidrug-resistance [20]. (4)Reversal effect of CUR on DNA repair mechanisms mediated Tumor multidrug-resistance [21].

Some researches confirmed that co-administration of CUR and DOX had synergic anti-cancer effect and the effect was dependent on the order and dosage of co-administration [22,23].In addition, the presence of CUR could also lower the cytotoxicity of DOX, therefore, the presence of CUR could increase the efficacy and decrease the side effects and the cytotoxicity of DOX and other traditional chemotherapeutic drugs when administered together. Given the above, the combination of CUR and chemotherapeutic drugs is a potential treatment in resolving the medical problems caused by the cytotoxicity and the multidrug resistance from long-term chemotherapy.

The effects of CUR on major multidrug-resistant factors has already been confirmed by researches, while whether these approaches can reverse drug resistance still need further study[24].Currently, the study on MDR reversal by CUR is just beginning. But the application of CUR is limited by many of its own-defects, such as water insolubility, poor absorption, instability and easy degradation. As more nanotechnologies are used in pharmaceutical science today, many defects and unsolvable problems in the past have been overcome. And more researches in recent years also suggest that NPs can effectively reverse multidrug resistance.

In this paper, co-encapsulated anti-cancer drug DOX and reversal agent CUR in PBCA-NPs were prepared with emulsion polymerization. The mean particle size and the zeta potential of DOX-CUR-PBCA-NPs were 133 ± 5.34nm in diameter and +32.23 ± 4.56 mV. The entrapment efficiencies of DOX and CUR were 49.98 ± 3.32% and 94.52 ± 3.14%, respectively. MTT assay and western blotting results shows that DOX-CUR-PBCA-NPs were equivalent to DOX-PBCA-NPs + CUR-PBCA-NPs in terms of inhibiting in vitro MCF-7/ADR cell growth and reversing P-gp mediated multidrug resistance in MCF-7/ADR. But DOX-CUR-PBCA-NPs consumed less amount of polymer. And the reversal efficacy of them were much higher than that of the free drug combination (DOX-CUR) and one free drug / another agent loaded PBCA-NPs combination (DOX+CUR-PBCA-NPs or CUR+DOX-PBCA-NPs). The reason maybe lie in the following mechanism: the overexpression of transmembrane pump P-gp increased DOX efflux and thus decreased DOX accumulation in cells, resulting in a higher resistant degree to anticancer agents; the drug loaded PBCA-NPs degraded into poly (cyan acrylic acid) on the cell membrane, and formed ion pairs with DOX to reduce drug efflux [25], which finally increased the drug accumulation and reversed MDR. CUR, the major active ingredient of a reversal agent curcuma, exhibited synergic reversal effects with DOX in the co-encapsulated NPs system, which led to a more pronounced reversal efficacy than single-drug loaded PBCA-NPs after 48h incubation.

The exact mechanism of reversing MDR by NPs system is not clear yet, but some scientists suggested that PBCA-NPs could stop the P-gp mediated transmembrane transport, which resulted in the increase of drug accumulation and cytotoxicity. ColindeVerdiere et al. [26] thought that DNR (daunorubicin)-PBCA-NPs could increase the drug accumulation in cells through the interaction between Nps and cells rather than through the endocytosis of cancer cells. Neamti et al. [27] have shown that PBCA-NPs absorbed on the cell surface could overcome MDR by preventing an immediate drug release and reducing endocytosis

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CONCLUSION

The data obtained in this study have shown that two complementary active drugs might be associated together with NPs using the emulsion polymerization technology. We have applied this concept to the cellular delivery of an anticancer compound (DOX) and P-gp inhibitor (CUR) to improve the efficacy of DOX NPs in overcoming MDR. The incorporation of DOX and CUR in the same nanoparticle formulation elicited the most effective growth rate inhibition compared to other alternative approaches, probably as a result of a synergistic effect due to the rapid release of a high amount of CUR at the surface of the cell membrane allowing a facilitated intracellular dilution of DOX. Therefore, our conclusion is that DOX-CUR-PBCA-NPs can strengthen the efficacy in overcoming MDR and can be expected to become a potential treatment with satisfactory in vivo efficacy.

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