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Characterization and cellular uptake of platinum anticancer drugs encapsulated in apoferritin

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ABSTRACT

Clinical application of platinum-based anticancer drugs is largely limited by severe general toxicity and drug resistance. Drug delivery systems with tumor-targeting potential are highly desired for improving the efficacy and applicability of these drugs. This study describes an alternative strategy for the delivery of platinum drugs (cisplatin, carboplatin and oxaliplatin) by encapsulating each of them in the cavity of apoferritin (AFt). The encapsulation was achieved through manipulating the pH-dependent unfolding–refolding process of AFt at pH 2.0 and 7.4, respectively, in saturated drug solution. UV–vis spectrometry, circular dichroism spectrometry, dynamic light scattering, and inductively coupled plasma mass spectrometry were used to characterize the AFt–drug complexes. The loading capacity of AFt varies with respective drugs and the structural integrity of the protein shell remains intact after encapsulation. In vitro assays on the rat pheochromocytoma cell line (PC12) show that AFt–cisplatin inhibits the cells in a slow but sustaining mode and the cellular uptake of platinum is enhanced by AFt. AFt–carboplatin and AFt–oxaliplatin complexes only exhibit a marginal cytotoxicity towards this cell line under similar concentrations.

1. Introduction

Platinum-based anticancer drugs such as cisplatin (CDDP), carboplatin (CBDCA) and oxaliplatin (LOHP) (Fig. 1) have been widely used to treat various solid tumors [1]. However, the therapeutic window of these drugs is drastically narrowed by their severe systemic toxicity and drug resistance from tumors [2]. For example, CDDP produces nephrotoxicity, neurotoxicity, ototoxicity and induces inherent and acquired drug resistance; CBDCA shows cross-resistance to CDDP and causes myelosuppression; and LOHP engenders cumulative sensory peripheral nerve damage [3,4]. The toxicities are believed to be derived from the interactions of platinum drugs with healthy tissues during the transmission and distribution of the drugs in the body [5]; and the drug resistance may be associated with insufficient or ineffective cellular uptake of the drugs [6]. Therefore, how to reduce the general toxicity and to improve the efficacy become the major goals in the development of platinum-based drugs.

Targeted drug delivery to tumor tissues could abate the side effects. The realization of this intention, however, is heavily dependent on suitable targeting carriers. Copolymer- [7] or liposomal-based [8,9] carriers have been used to improve the delivery of platinum drugs for the enhanced permeability and retention (EPR) effect of macromolecules on tumors, but altered tissue distribution would lead to new toxicity profiles in clinical trials [10]. Recently, molecular containers with large cavities such as cucurbituril series were examined as drug delivery vehicles for platinum complexes [11], but poor water solubility and inefficient transport limit their potential application [12]. As an alternative, ligand–receptor-mediated delivery systems have attracted much attention because of their non-immunogenic and site-specific targeting potential to the ligand-specific bio-sites [13]. For instance, transferrin has been exploited for the delivery of CDDP into proliferating malignant cells that over-express transferrin receptors [14]. On the same score, biomolecules such as folic acid [15,16], estrogen [17], herceptin [18], and galactose residues [19,20] have also been incorporated into hybrid complexes to enhance the targeting property of the drugs towards tumor tissues with respective receptors. In our recent work, the demineralized ferritin, i.e. apoferritin (AFt), has been shown to be a promising vehicle for targeted delivery of platinum-based drugs [21]. AFt is a hollow cage with internal and external diameters of 8 and 12 nm, respectively [22]. Since ferritin-binding sites [23] and endocytosis of ferritin [24] have been identified in neoplastic cells, and receptors of ferritin have shown some potential in the delivery of anticancer drugs into the brain [25], AFt may enhance the drug selectivity for cell surfaces that express ferritin receptors.

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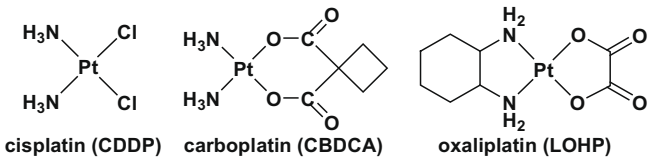


Fig. 1. Platinum-based anticancer drugs used in this work.

In this paper, we have extended our previous work to a wider range where LOHP is also involved in the encapsulation by AFt, and enriched the means of characterization for the AFt–drug complexes. More importantly, the dose-responsive and time-dependent cytotoxic profiles and the enhanced cellular uptake of AFt–CDDP complexes are observed against rat pheochromocytoma cell line (PC12). The three AFt–drug complexes are denoted hereafter as AFt–CDDP, AFt–CBDCA, and AFt–LOHP, respectively.

2. Experimental

2.1. Materials

Ferritin was purchased from Sigma. CDDP, CBDCA and LOHP were obtained from Shandong Boyuan Chemical Co., Ltd. Other chemical reagents were used as received without further purification. Doubly deionized water (18.2 MΩ cm at 25 °C) prepared on a Milli-Q (MQ) water system was used throughout all experiments.

2.2. Encapsulation of platinum drugs

AFt was obtained from the horse spleen ferritin by demineralization according to the established procedures [26]. The encapsulation of platinum-based drugs in the AFt cavity was carried out as described previously [21]. Briefly, the platinum drugs were dissolved, respectively, to get their saturated solutions (CDDP, 1 mg mL^{-1}; CBDCA, 15 mg mL^{-1}; LOHP, 5 mg mL^{-1}). AFt was added to each solution to reach a final protein concentration of 1 mg mL^{-1}. The dissociation of AFt into its subunits was induced by adjusting the pH of the mixed solution to 2.0 with HCl (1 M) and then maintaining this value for about 15 min. Afterwards, the pH was slowly adjusted back to 7.4 using NaOH (1 M). The resulting solution was stirred at 100 rpm under room temperature for 2 h and dialyzed against saline water (NaCl, 0.15 M, renewed three times) for 24 h to completely remove drug molecules outside of the protein shell. After that, the solution was centrifuged at 10,000 rpm for 10 min to remove the precipitates. The solutions of AFt–drug complexes were concentrated using Amicon Ultra-15 centrifugal filters (MWCO 50, Millipore, Bedford, MA). The concentration of AFt was determined in triplicate by the BCA protein assay kit (Pierce, Socachim, Switzerland) using bovine serum albumin as the standard.

2.3. Characterization of AFt and AFt–drug complexes

AFt and AFt–drug complexes were characterized by the following measurements. UV–vis absorption spectra were performed on a Shimazu UV-3100 spectrometer supplied with Perkin Elmer UV WinLab (version 1.1) computer software. Circular dichroism (CD) spectra were recorded on a Jasco J-810 spectropolarimeter in the far-ultraviolet wavelength range of 190–250 nm in a quartz cell (0.1 cm) using following parameters: bandwidth, 1 nm; step resolution, 0.1 nm; scan speed, 10 nm min^{-1}; and response time, 1 s. The wavelength and optical rotation of the instrument have been calibrated by benzene vapor and d-10-camphorsulphonic acid, respectively. The data of each spectrum were the average of three scans. All samples were at the same protein concentration (0.5 mg mL^{-1}, 0.1 M NaCl). Hydrodynamic diameters were determined using a BI-200SM dynamic light scattering (DLS, Brookhaven Instruments Co., Holtsville, NY). The protein concentration of the samples is *ca.* 0.1 mg mL^{-1} in 0.1 M NaCl. All samples were filtered through a 0.45 μm filter before analysis and the average of triplicate values was adopted. The data were analyzed by the 9kdlsw_v3.50 software. Zeta potential (ζ) of AFt and AFt–drug complexes was measured in NaCl (0.1 M) with the protein concentration of *ca.* 0.1 mg mL^{-1} on a Malven Nano-Z instrument. The mean of triplicate measurements is taken as the final result.

2.4. Pt analysis

Pt analysis was performed on inductively coupled plasma mass spectrometer (ICP-MS) using a standard Plasma-Quad II instrument (VG Elemental, Thermo Optek Corp.). A three-point calibration curve was made for all measurements against Pt-containing solutions prepared by serial dilution of a certified reference standard. The most abundant isotope of Pt was monitored at m/z 195 [27]. The calibration was linear (typical $r > 0.999$) over the analytical working range (0.1–100 μg L^{-1}). The samples were diluted by 100-fold to make the final Pt concentration within the working range. The nitrolysis of the samples was carried out sequentially with concentrated HNO_3 at 95 °C for 2 h, H_2O_2 at 95 °C for 1.5 h and concentrated HCl at 37 °C for 0.5 h. Finally, the solution was diluted to 2 mL with MQ water and the Pt content was measured. The reported result of the sample is the average of three replicates. A Pt-free solution prepared likewise was used as a control in each test to detect any possible contamination; and in most cases the Pt content was below 0.5% of that in the samples. The Pt content in the final dialytic solutions was also determined to ensure the exhaustive dialysis.

2.5. Cytotoxicity assay

Growth inhibitory effect of the AFt–drug complexes on PC12 cells was tested by the MTT assay [28]. PC12 cells were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with fetal bovine serum (10%, v/v), streptomycin (0.1 mg mL^{-1}) and penicillin (100 U mL^{-1}) in a humidified atmosphere with 5% CO_2 at 37 °C. The cells were seeded in 96-well plates at 5×10^3 cells per well in DMEM medium and incubated overnight, which were then treated in triplicate with fresh medium containing grade concentrations (on Pt) of AFt–drug complex with free drug and AFt as the references. The cells in the plates were incubated at 37 °C under 5% CO_2 for 72 h and were then incubated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 10 μL) solution (5 mg mL^{-1}) in PBS buffer (8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4 per liter) for 4 h. DMSO (150 μL) was added to each well after the medium was removed. The absorbance of the purple formazan was recorded at 490 nm using an ELISA plate reader. The cytotoxicity results were calculated based on the data of three replicate tests. The time-dependent growth inhibitory effect of AFt–CDDP on PC12 cells were carried out similarly at the IC_{50} concentration of AFt–CDDP (20 μM).

2.6. Cellular uptake

PC12 cells were seeded into a 12-well plate at 10^5 cells per well and incubated overnight. The medium was refreshed (1 mL per well) and the cells were treated with AFt–CDDP and CDDP, respectively, at the IC_{50} concentration of AFt–CDDP (20 μM) and incubated at 37 °C for 24 h. The medium was removed and the cells were incubated with trypsin solution for 1 min. After digestion,

the cells were rinsed with PBS (1 mL × 3). Cell numbers were counted using trypan blue staining and the harvested cells were dispersed in ultrapure water (50 μL) and the nitrolysis was carried out as described above. The resulting solution was diluted to 1.2 mL and the Pt content was determined using ICP-MS. The average value of three independent experiments was taken as the final value for Pt uptake.

3. Results and discussion

3.1. Characterization of AFt–drug complexes

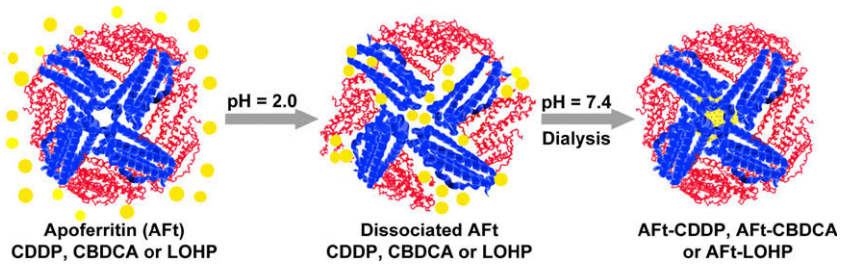
Encapsulation of platinum-based anticancer drugs in AFt was completed by two steps as reported previously [29]. Firstly, platinum drugs were entrapped in the cavity of AFt via a pH-induced protein unfolding–refolding process in the presence of their respective saturated solutions; secondly, AFt–drug complexes were separated, respectively, from the remaining free drug molecules outside of the protein shell by exhaustive dialysis (Scheme 1).

The UV spectra of the AFt–drug complexes were determined firstly to examine whether the structure of AFt has been changed after the encapsulation of platinum drugs. As shown in Fig. 2A, except for the variations in absorbance intensity, all AFt–drug complexes and AFt exhibit similar spectral features with their characteristic absorption appearing at 280 nm. This absorption arises from the amino acid residues with aromatic rings in AFt; and its intensity correlates linearly with the concentration of protein [30]. The results suggest that AFt–drug complexes have similar structures to that of AFt and encapsulation of platinum drugs in the protein cavity barely affects the surface nature of AFt.

The CD spectra in the far-ultraviolet region were recorded to further ascertain any possible changes in the secondary structure of AFt after the encapsulation of platinum drugs [31]. Fig. 2B shows the CD spectra of AFt–drug complexes and AFt in the range of 190–250 nm, but they nearly overlap each other. It is known that ferritin cages are formed by arrays of self-assembling α-helices [32]. The quantitative evaluation made by the CDPro software package (data not shown) indicates that the content of α-helix in AFt almost remains constant before and after the encapsulation. The above UV and CD results demonstrate that the structure of AFt keeps intact after loading with platinum drugs even if AFt underwent a dramatic pH change during the unfolding–refolding process. In the circumstances, the targeting potential of AFt to its receptor may not be impaired in AFt–drug complexes.

Further evidence for the integrity of AFt structure was provided by DLS experiments. The hydrodynamic diameters (Φ) of AFt and AFt–drug complexes determined by DLS are indicated in Fig. 3. AFt has an average diameter of 12.3 nm, which is in agreement with the previous report [22]. The average diameters of AFt–CDDP, AFt–CBDCA, and AFt–LOHP are 12.5, 13.0, and 12.6 nm, respectively. The size differences among AFt and AFt–drug complexes are negligible, suggesting AFt is still properly assembled after the encapsulation of drugs. The moderate size of these AFt–drug complexes may facilitate their smooth circulation in the body.

Zeta potential (ζ) is an indication of surface charges on a particulate species, which can profoundly affect particle distribution, cellular uptake and surface binding *in vivo* [33]. Table 1 presents the zeta potential of AFt and AFt–drug complexes with respective experimental errors. AFt (*ca.* 0.1 mg mL^{-1}, 0.1 M NaCl) exhibits a potential of −18.2 mV; in the same condition, AFt–CDDP, AFt–CBDCA, and AFt–LOHP also show similar potential values. The re-



Scheme 1. Schematic illustration of the pH-mediated encapsulation of cisplatin (CDDP), carboplatin (CBDCA), or oxaliplatin (LOHP) by apoferritin (AFt) via an unfolding–refolding process.

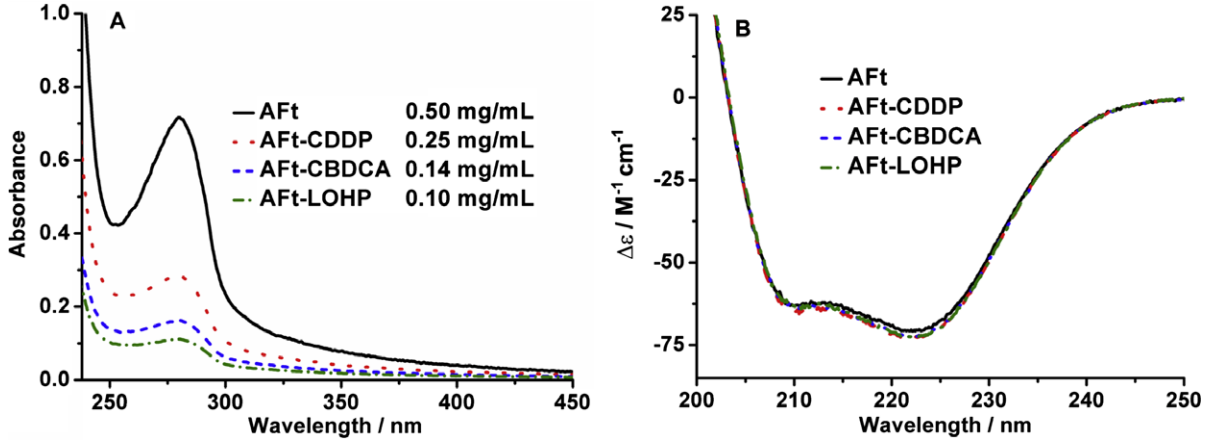


Fig. 2. UV–vis (A) and far-UV CD (B) spectra of apoferritin (AFt) before and after the encapsulation of cisplatin (CDDP), carboplatin (CBDCA), or oxaliplatin (LOHP). UV spectra were recorded at different protein concentrations; while CD spectra were recorded at the same protein concentration (0.5 mg mL^{-1}, 0.1 M NaCl).

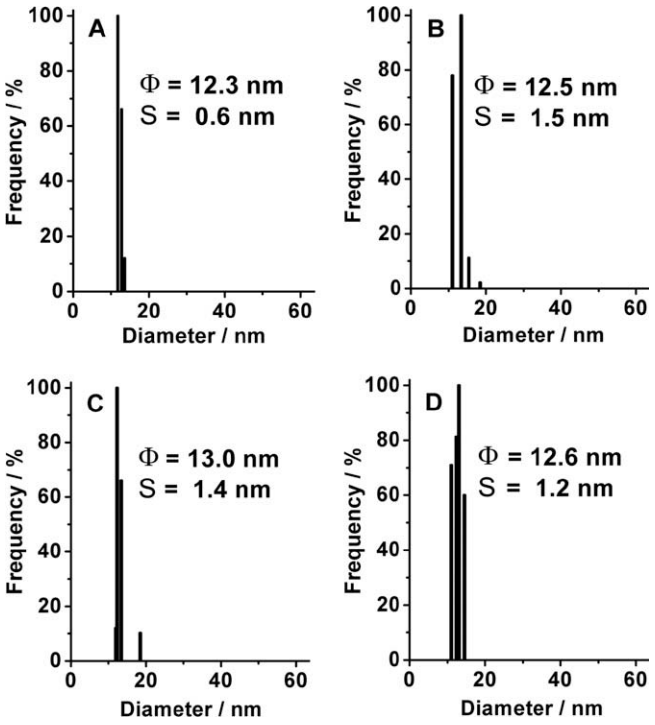


Fig. 3. Size distribution of AFt (A), AFt–CDDP (B), AFt–CBDCA (C), and AFt–LOHP (D) determined by dynamic light scattering (DLS) at the same protein concentration (*ca.* 0.1 mg mL^{-1}, 0.1 M NaCl). *Φ*: Average diameter; *S*: standard deviation of size distribution.

Table 1
Zeta potential of AFt, AFt–CDDP, AFt–CBDCA, and AFt–LOHP recorded at the same AFt concentration (*ca.* 0.1 mg mL^{-1}, 0.1 M NaCl).

Sample	AFt	AFt–CDDP	AFt–CBDCA	AFt–LOHP
Zeta potential (mV)	−18.2 ± 0.5	−16.6 ± 0.4	−17.4 ± 0.1	−18.3 ± 0.4

sults suggest that the electrostatic property on the surface of AFt hardly changed after platinum drugs were enwrapped in its cavity, and hence the binding affinity of AFt–drug complexes for some target receptors should remain unchanged.

3.2. Pt analysis

Platinum drug molecules entrapped in every AFt cage were determined by ICP-MS and BCA methods. At least six independent samples were examined for each AFt–drug complex to evaluate the encapsulation efficiency. Table 2 lists the concentrations of Pt and AFt for three AFt–drug complexes. The molar ratio of Pt to AFt for AFt–CDDP, AFt–CBDCA, and AFt–LOHP are 45, 17, and 23, respectively. The stability of the AFt–drug complexes was also verified by ICP after dialysis for different periods of time (24–72 h). The Pt content with respect to AFt is almost independent on time. Based on the detailed data of ICP and BCA in Table 2, it can be concluded that the reproducibility of these experiments is quite good, and the encapsulation efficiency of AFt for platinum drugs has been improved significantly as compared with what we reported previously [21]. The relative high loading capacity of AFt enables it to carry enough drug molecules required for the cytostatic function.

3.3. Cytotoxicity

The antitumor potential of AFt–drug complexes was tested against the rat pheochromocytoma (PC12) cell line with AFt and respective free drugs as controls. Fig. 4 displays the concentration-dependent cellular viability in the presence of AFt–drug complexes determined by the MTT assay after 72 h. AFt–CDDP shows a considerable cytotoxicity as compared with that of AFt, but it is inferior to CDDP at the same Pt concentration. The cellular viability of PC12 cells is negatively correlated with the concentrations of AFt–CDDP and CDDP (Fig. 4A). The IC_{50} values for AFt–CDDP and CDDP are 20 and 2 μM, respectively, indicating the former is less cytotoxic than the latter; however, they are almost equally effective against PC12 cells at high Pt concentrations (>100 μM). A much less inhibition is observed for AFt–LOHP as compared with LOHP (IC_{50} = 4 μM) even if the concentration reaches to 10 μM (Fig. 4B). AFt–CBDCA and CBDCA exhibit insignificant cytotoxicity as that of AFt under the experimental concentrations. The limited release of drug molecules from the protein cavity may account for the unremarkable cytotoxicity of these AFt–drug complexes. On the other hand, CBDCA and LOHP are intrinsically less cytotoxic than CDDP and require longer time for cytosolic activation to exert the cytotoxic effect [34,35]; therefore, AFt–CBDCA and AFt–LOHP complexes are not as effective as AFt–CDDP in this study. Although much antitumor activity of CDDP is retained in AFt–CDDP, the cytotoxic profile has been changed, which might affect the *in vivo* pharmacological behavior of the drug [34], but further experiments are needed to verify this presumption.

Table 2
The molar ratios of Pt to AFt based on the encapsulated Pt content and AFt concentration for each AFt–drug complex.

Sample	Pt (μg L^{-1})[a]		AFt (μg mL^{-1})[b]		Pt/AFt	
	Individual	Average	Individual	Average	Individual	Average
AFt–CDDP	4588	4864.1	204	249.2	51	45 ± 4
	4242		240		40	
	4070		221		42	
	5690		282		46	
	5403		272		45	
	5193		276		43	
AFt–CBDCA	1007	972.5	145	134.3	16	17 ± 1
	1025		147		16	
	965		123		18	
	791		101		18	
	877		132		15	
	1170		158		17	
AFt–LOHP	884	999.7	89	95.8	22	23 ± 1
	985		100		22	
	1060		99		24	
	1055		96		25	
	1012		95		24	
	1002		96		23	

[a] Determined by ICP-MS method.
[b] Examined by BCA assay.

3.4. Cellular uptake

The cellular uptake status of Pt by PC12 cells was determined by ICP-MS. As shown in Table 3, the Pt uptake associated with AFt–CDDP and CDDP is 0.085 and 0.019 nmol per 10^5 cells, respectively. The Pt uptake of AFt–CDDP is much higher (*ca.* 4.5-fold) than that of CDDP, which suggests that AFt–CDDP can be more readily internalized by the tumor cells. Similar phenomena were also observed in the macromolecule-based platinum drug delivery system [34] and the mechanism of internalization was believed to be via endocytosis [7]. The enhanced cellular uptake of AFt–CDDP may also result from the higher affinity of iron for AFt, because iron is often rich in neoplastic and nerve cells [23]. Since reduced cellular uptake is a major mechanism for the resistance to platinum drugs [1], the enhanced uptake of AFt–CDDP is particularly significant for defeating the lingering CDDP resistance.

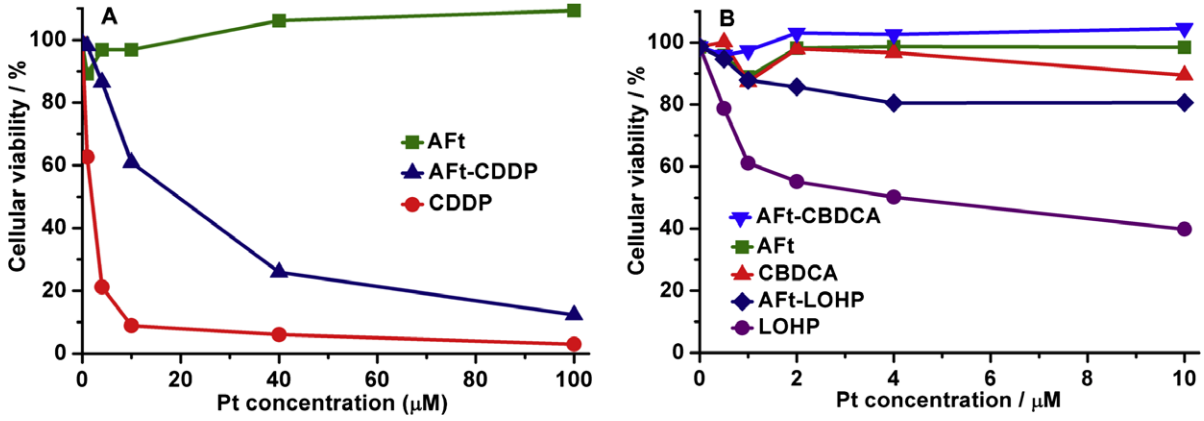


Fig. 4. Cytotoxic profiles of AFt–drug complexes determined by the MTT assay on PC12 cell line after 72 h with AFt and respective free drugs as the references: (A) AFt–CDDP; (B) AFt–CBDCA and AFt–LOHP.

Table 3
Cellular uptake of Pt associated with AFt–CDDP and CDDP by PC12 cells during 24 h.

Sample	Initial Pt (nmol 10^{-5} cells)	Pt uptake (nmol 10^{-5} cells)	Uptake ratio (%)
AFt–CDDP	20	0.085	0.425
CDDP	20	0.019	0.095

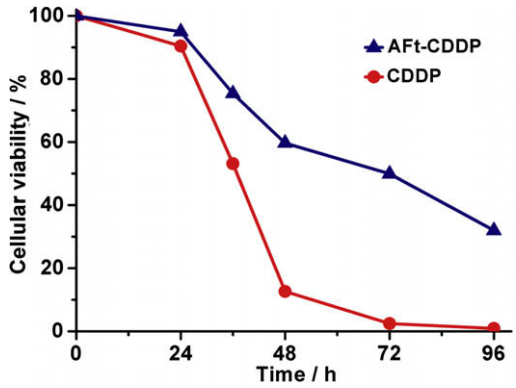


Fig. 5. Time-dependent growth inhibition profiles of AFt–CDDP and CDDP against PC12 cells at the IC_{50} concentration of AFt–CDDP (20 μM).

A time-dependent cytostatic activity of AFt–CDDP and CDDP was assayed against PC12 cells to find some pharmacokinetic differences between them. As Fig. 5 shows, the cytotoxicity of AFt–CDDP is lower than CDDP over the whole incubation time. Moreover, AFt–CDDP exerts the cytotoxic effect in a slow and ever-increasing manner; but CDDP acts in a fast way in that the inhibition climax is nearly reached at 48 h. The cytotoxic effect of AFt–CDDP is milder and slower than that of CDDP because AFt–CDDP needs to release the enclosed CDDP from the protein cage to suppress cells, and the release might be a slow and incomplete process. To keep drug molecules inactive during circulation and to release them timely at the target site are the essential requirements for an ideal carrier [6]. Apparently, AFt as a carrier meets these criteria and hence could be a prospective vehicle for the delivery of platinum drugs. However, the release mechanism of platinum drugs from the AFt–drug complexes is not yet clear. It is known that the microenvironment of tumors is slightly acidic with pH value being 0.5–1.0 units lower than that in normal tissues [36], which might be a stimulus to the release process. After all, the release of iron from ferritin is pH-dependent and low pH facilitates the release [37]; similar events may occur for the release of platinum drugs from AFt cages.

4. Conclusions

High systemic toxicity and severe drug resistance are two major defects of platinum-based anticancer drugs. The development of targeted drug delivery system has made some encouraging advances in solving these problems. Among many smart strategies in this area, ligand-receptor-mediated delivery system is the most preferred model. In this study, we use apoferritin (AFt) as a carrier to encapsulate three platinum anticancer drugs, cisplatin (CDDP), carboplatin (CBDCA) and oxaliplatin (LOHP), into its cavity, and thereby endow these drugs with some tumor targeting property. The structural integrity of AFt is well preserved in the AFt–drug complexes, and the potential homing groups on the surface of AFt are not affected by the encapsulation. Since the binding sites and endocytosis of ferritin have been identified in some tumor cells, platinum drugs in AFt could be delivered selectively to tumor cells that over-express ferritin receptors. More importantly, AFt–CDDP shows a significant increase in cellular uptake as compared with CDDP, which suggests that CDDP-related drug resistance might be overcome by this complex. In short, tumor targeting potentiality, relative high loading capacity and good water solubility make AFt a promising carrier for platinum-based anticancer drugs.

5. Abbreviations

AFt	apoferritin
CDDP	cisplatin
CBDCA	carboplatin
LOHP	oxaliplatin
DLS	dynamic light scattering
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DMEM	Dulbecco's modified Eagle's medium
IC_{50}	half maximal inhibitory concentration
BCA	bicinchoninic acid
ELISA	enzyme-linked immunosorbent assay
EPR	enhanced permeability and retention
ICP-MS	inductively coupled plasma mass spectrometry
PBS	phosphate buffered saline

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30870554) and the Natural Science Foundation of Jiangsu Province (BK2008015) is gratefully acknowledged.

References

[1] D. Wang, S.J. Lippard, Nat. Rev. Drug Discov. 4 (2005) 307–320.
[2] X.Y. Wang, Z.J. Guo, Dalton Trans. (2008) 1521–1532.
[3] C.A. Rabik, M.E. Dolan, Cancer Treat. Rev. 33 (2007) 9–23.
[4] A.A. Argyriou, P. Polychronopoulos, G. Iconomou, E. Chroni, H.P. Kalofonos, Cancer Treat. Rev. 34 (2008) 368–377.
[5] S. van Zutphen, J. Reedijk, Coord. Chem. Rev. 249 (2005) 2845–2853.
[6] L. Kelland, Nat. Rev. Cancer 7 (2007) 573–584.
[7] J.R. Rice, J.L. Gerberich, D.P. Nowotnik, S.B. Howell, Clin. Cancer Res. 12 (2006) 2248–2254.
[8] S.C. White, P. Lorigan, G.P. Margison, J.M. Margison, F. Martin, N. Thatcher, H. Anderson, M. Ranson, Br. J. Cancer 95 (2006) 822–828.
[9] K.N.J. Burger, R.W.H.M. Staffhorst, H.C. de Vijlder, M.J. Velinova, P.H. Bomans, P.M. Frederik, B. de Kruijff, Nat. Med. 8 (2002) 81–84.
[10] D.N. Waterhouse, P.G. Tardi, L.D. Mayer, M.B. Bally, Drug Saf. 24 (2001) 903–920.
[11] A.M. Krause-Heuer, M.P. Grant, N. Orkey, J.R. Aldrich-Wright, Aust. J. Chem. 61 (2008) 675–681.
[12] N.J. Wheate, R.I. Taleb, A.M. Krause-Heuer, R.L. Cook, S. Wang, V.J. Higgins, J.R. Aldrich-Wright, Dalton Trans. (2007) 5055–5064.
[13] S.P. Vyas, V. Sihorkar, Adv. Drug Deliv. Rev. 43 (2000) 101–164.
[14] H. Iinuma, K. Maruyama, K. Okinaga, K. Sasaki, T. Sekine, O. Ishida, N. Ogiwara, K. Johkura, Y. Yonemura, Int. J. Cancer 99 (2002) 130–137.
[15] E. Gabano, M. Ravera, C. Cassino, S. Bonetti, G. Palmisano, D. Osella, Inorg. Chim. Acta 361 (2008) 1447–1455.
[16] O. Aronov, A.T. Horowitz, A. Gabizon, D. Gibson, Bioconjug. Chem. 14 (2003) 563–574.
[17] A. Jackson, J. Davis, R.J. Pither, A. Rodger, M.J. Hannon, Inorg. Chem. 40 (2001) 3964–3973.
[18] J. Gao, Y.G. Liu, R.B. Liu, R.A. Zingaro, ChemMedChem 3 (2008) 954–962.
[19] Y. Ohya, K. Nagatomi, T. Ouchi, Macromol. Biosci. 1 (2001) 355–363.
[20] Y. Ohya, H. Oue, K. Nagatomi, T. Ouchi, Biomacromolecules 2 (2001) 927–933.
[21] Z. Yang, X.Y. Wang, H.J. Diao, J.F. Zhang, H.Y. Li, H.Z. Sun, Z.J. Guo, Chem. Commun. (2007) 3453–3455.
[22] M. Uchida, M.T. Klem, M. Allen, P. Suci, M. Flenniken, E. Gillitzer, Z. Varpness, L.O. Liepold, M. Young, T. Douglas, Adv. Mater. 19 (2007) 1025–1042.
[23] J.M. Pasquini, B. Aires (Eds.), Dev. Neurosci. 24 (2002) 94–251.
[24] M.S. Bretscher, J.N. Thomson, EMBO J. 2 (1983) 599–603.
[25] S.W. Hulet, S. Powers, J.R. Connor, J. Neurol. Sci. 165 (1999) 48–55.
[26] S. Stefanini, S. Cavallo, C.-Q. Wang, P. Tataseo, P. Vecchini, A. Giartosio, E. Chiancone, Arch. Biochem. Biophys. 325 (1996) 58–64.
[27] K. Benkhedda, B. Dimitrova, H.G. Infante, E. Ivanova, F.C. Adams, J. Anal. Atomic Spectrom. 18 (2003) 1019–1025.
[28] J.Y. Zhang, X.Y. Wang, C. Tu, J. Lin, J. Ding, L.P. Lin, Z.M. Wang, C. He, C.H. Yan, X.Z. You, Z.J. Guo, J. Med. Chem. 46 (2003) 3502–3507.
[29] B. Webb, J. Frame, Z. Zhao, M.L. Lee, G.D. Watt, Arch. Biochem. Biophys. 309 (1994) 178–183.
[30] C.M. Stoscheck, Methods Enzymol. 182 (1990) 50–68.
[31] J. Swift, W.A. Wehbi, B.D. Kelly, X.F. Stowell, J.G. Saven, I.J. Dmochowski, J. Am. Chem. Soc. 128 (2006) 6611–6619.
[32] X. Liu, E.C. Theil, Acc. Chem. Res. 38 (2005) 167–175.
[33] C.J. Sunderland, M. Steiert, J.E. Talmadge, A.M. Derfus, S.E. Barry, Drug Dev. Res. 67 (2006) 70–93.
[34] O. Aronov, A.T. Horowitz, A. Gabizon, M.A. Fuertes, J.M. Pérez, D. Gibson, Bioconjug. Chem. 15 (2004) 814–823.
[35] O. Rixe, W. Ortuzar, M. Alvarez, R. Parker, E. Reed, K. Paull, T. Fojo, Biochem. Pharmacol. 52 (1996) 1855–1865.
[36] I.F. Tannock, D. Rotin, Cancer Res. 49 (1989) 4373–4384.
[37] M. Hoppler, A. Schönbächler, L. Meile, R.F. Hurrell, T. Walczyk, J. Nutr. 138 (2008) 878–884.